HIV vaccines, neutralising antibodies, ADCC and beyond

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Viro-Immunobabble bingo

- Virology/Immunology/vaccinology full of jargon!

Streptavidin	SHIV	Vpu	Bystander killing	Immunity
bNab	Protection	Immunopathogenesis	Nef	TZMBL
CD4-induced	ADP	IMMUNOBABBLE (free square)	Viral load	Viral inhibition assay
Transmission	Elispot	Reproducibility	RV144	Fc receptor
Reservoir	Presentation	Polyfunctional	Trm	Urgent

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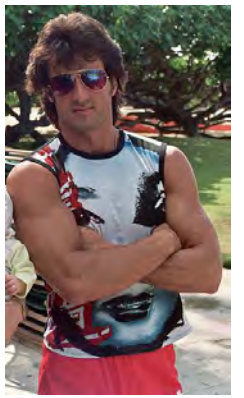
- Shout “ Immunobabble “

Prizes!

- Wine
- Chocolates
- And more

HIV vaccine immunology – a personal historical view

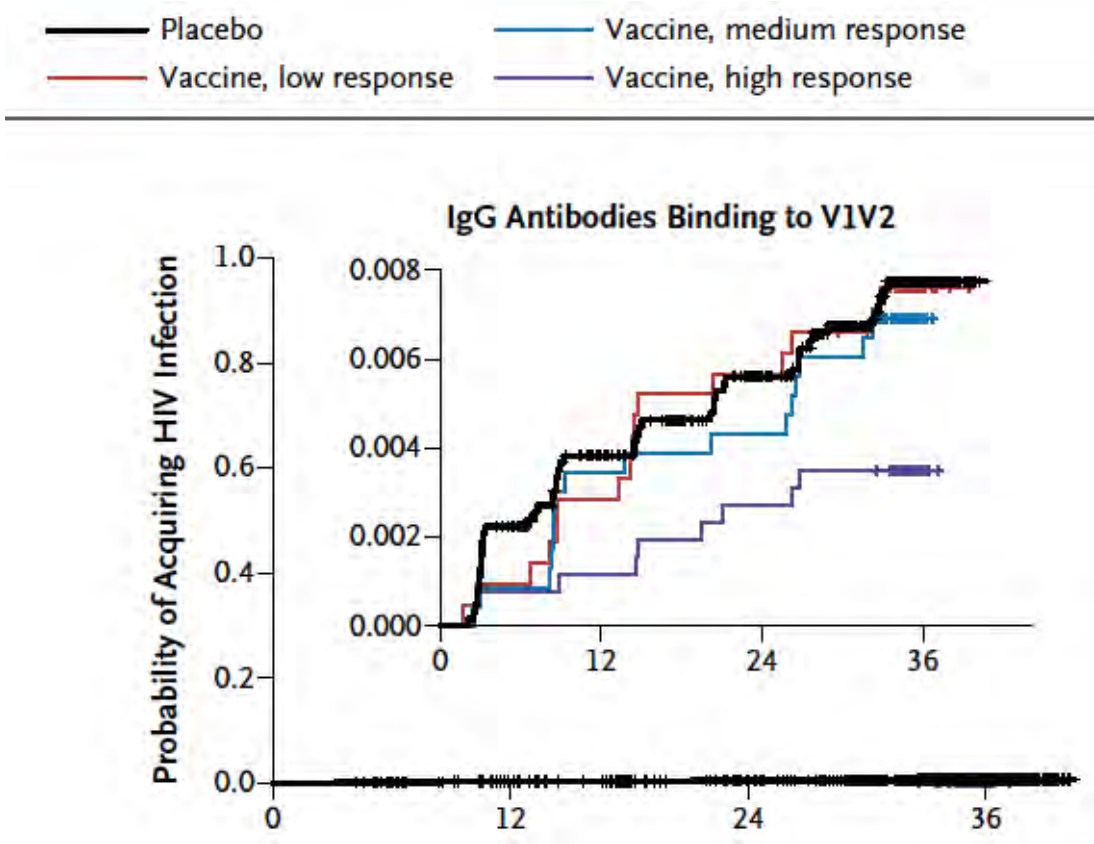


- 1980s – early 1990s – **Neutralizing Abs were king!**
  - Initial viruses were lab-adapted X4, relatively easy to neutralise
  - Emerging sense that field isolates would be so hard to neutralise
  - Dethroned with Vaxgen trials
- Mid 1990s – **Live attenuated vaccines are king!**
  - Humans with nef deleted viruses, impressive SIV protection studies
  - Dethroned with safety data.
- 1990s – mid 2000s – **CTLs were king!**
  - Strong evidence for control of infection
  - Improvement in viral vectors/prime boost etc
  - Escape and HLA restriction minor annoyances
  - Dethroned with STEP/Phambili and later HVTN505 studies
  - Better vectors, better inserts on the march now

HIV vaccine immunology – a personal historical view

- Mid-Late 2000s
  - Field wallowing. “Vector mania”. More and more BnAbs coming through.
  - Initiation of RV144 trial heavily criticized.

- 2009
  - RV144 shows weak efficacy signal.
  - Supported by correlates and sieving studies.
  - Non-neutralizing antibodies with Fc-mediated functions implicated.
  - Bnabs with ADCC function protect monkeys better.
  - **ADCC is king!**
  - Perhaps until partially dethroned by actually inducing decent Nabs!



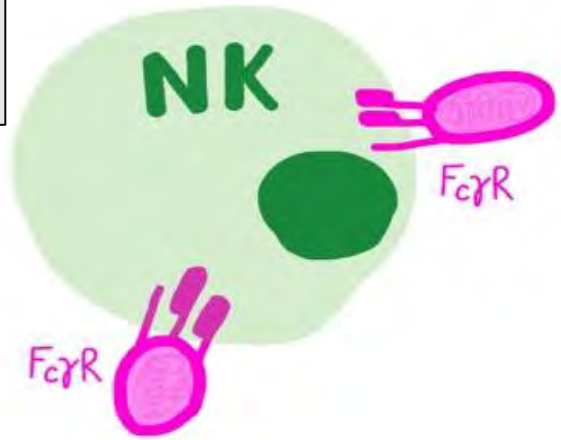
What drove the field? – technologies to readily and reliably measure responses

- Neutralizing antibodies:
  - **Cell line (TZMbl) entry assay,** rigorous panels of viruses, tiered viruses, positive control bNabs, IC50s – correlate well with protection
- CTLs:
  - **ELISpot, ICS assays**
  - Endless T cell function assays…
  - Viral Inhibition assays…
- ADCC
  - ^{51}Cr release assays…. Flow-based "killing" assays…. Antibody-dependent cellular viral inhibition (ADCVI) assay… Luciferase-based killing assay…. NK cell activation assays (ICS)….
  - Most require donor effector cells, difficult to standardise across labs, low throughput – has limited the field.

What is ADCC?

Fc-mediated functions

FcgRIIIa = CD16a
"ADCC FcR"

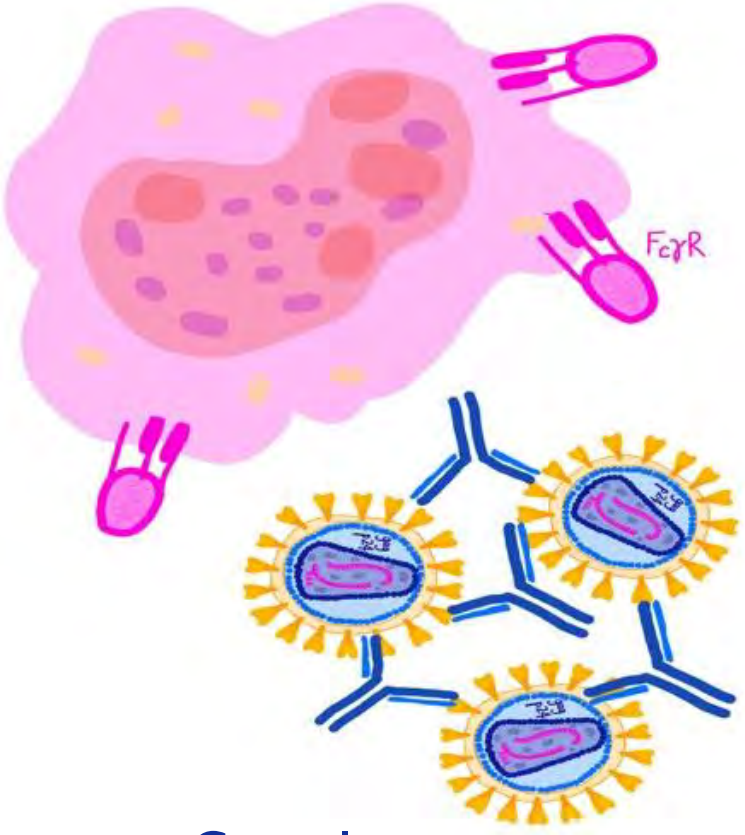


Natural Killer Cell

Infected Cell

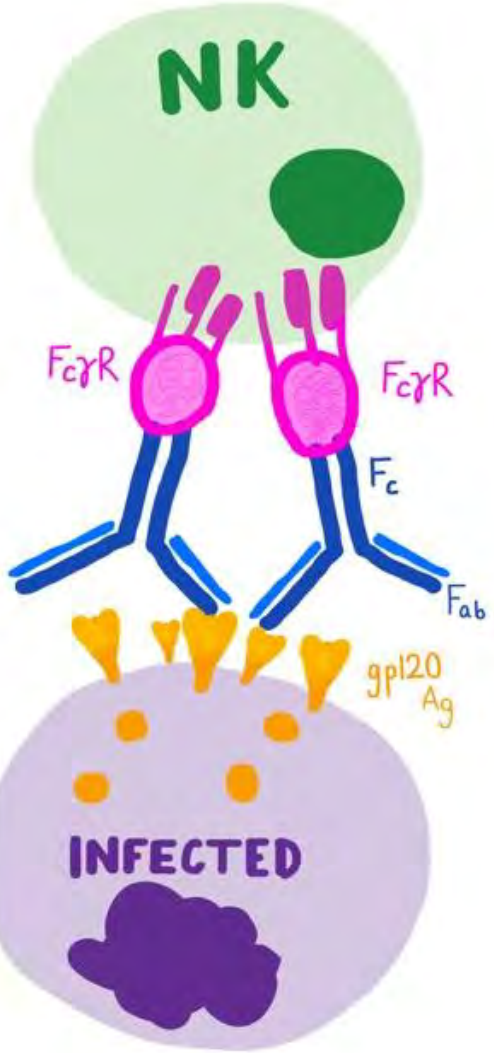
FcgRIIa = CD32a
"Phagocytosis FcR"

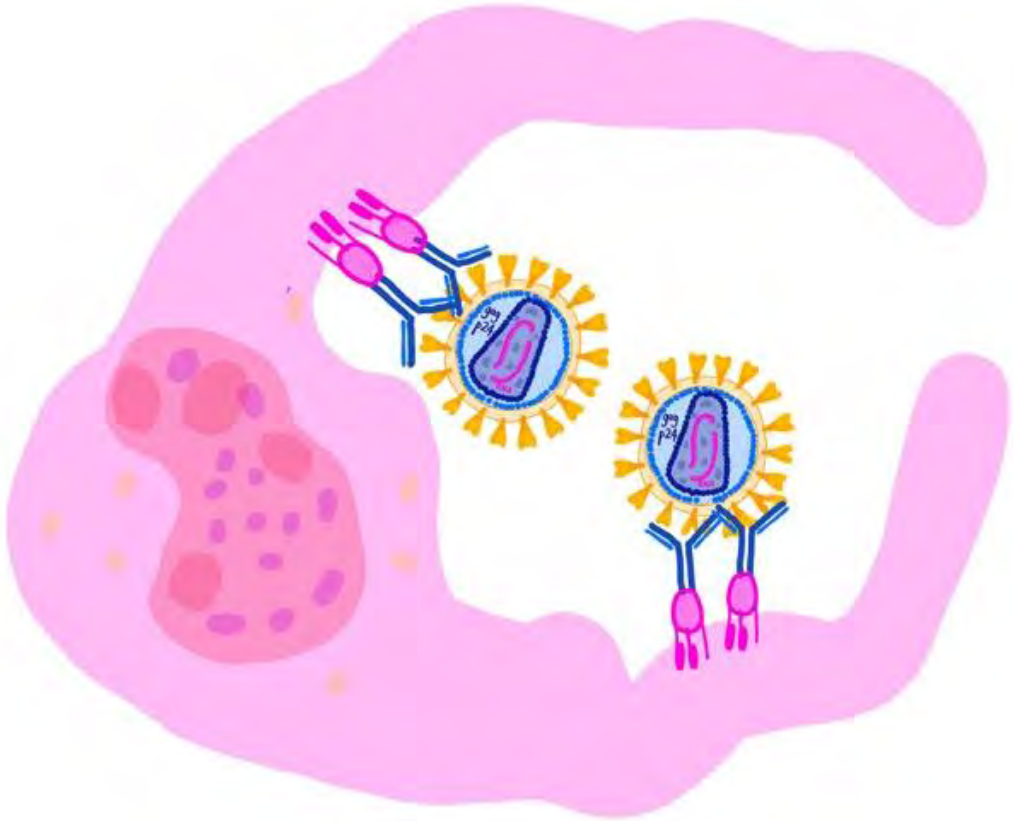
Macrophage

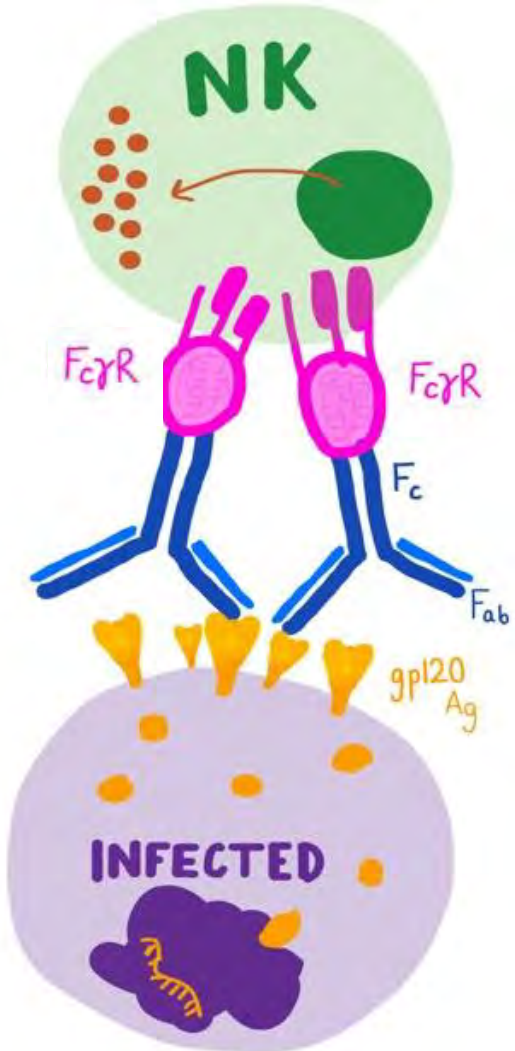


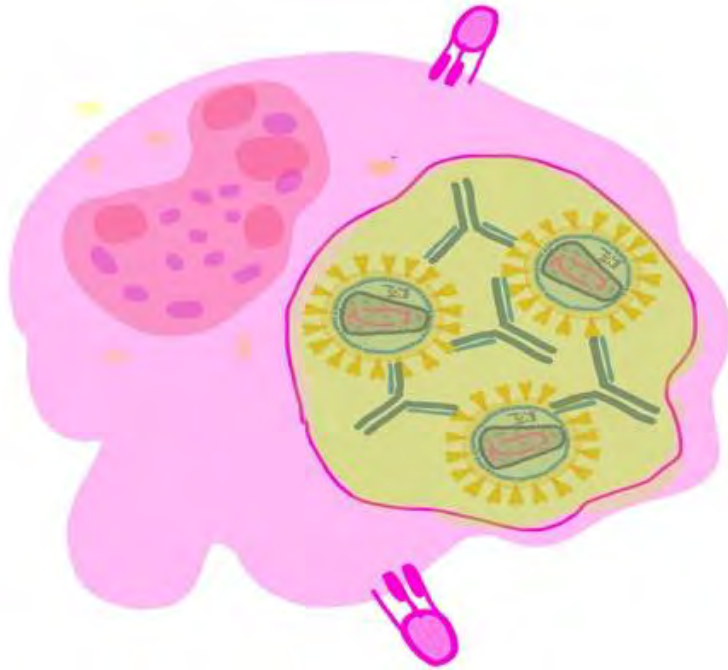
Immune Complex

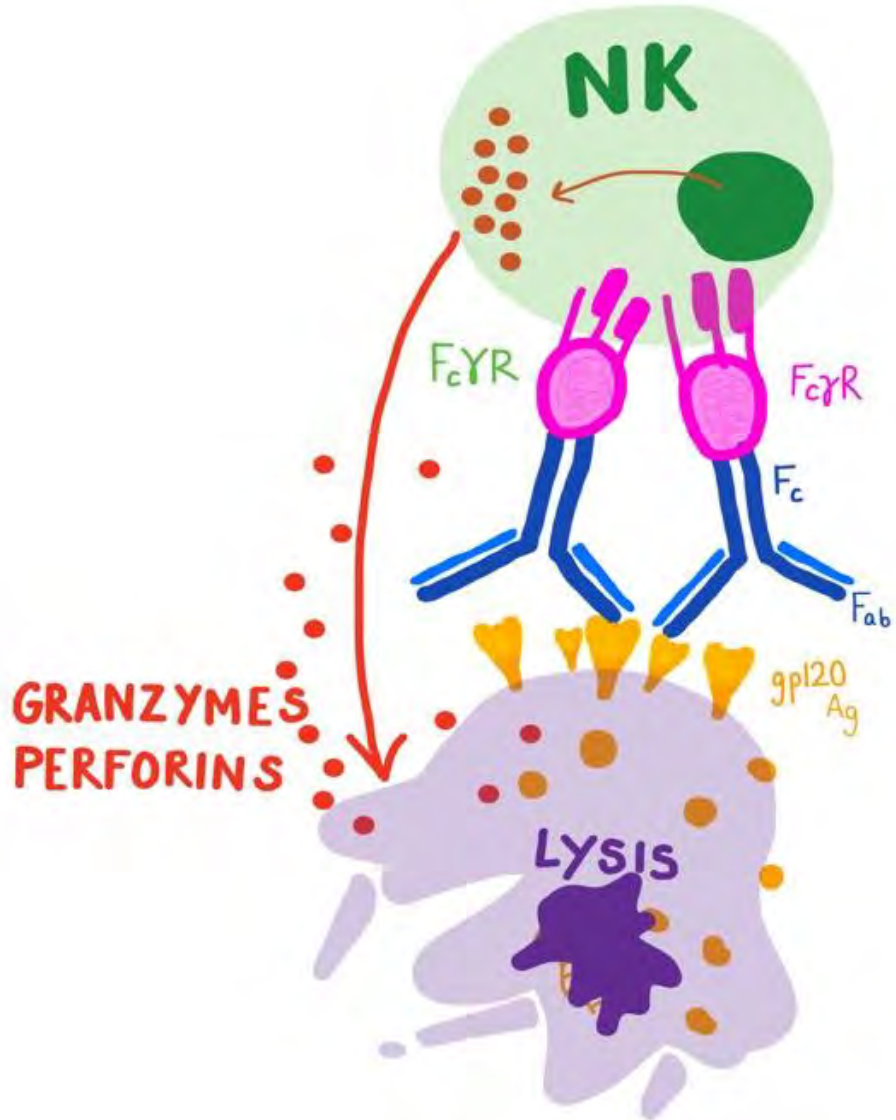
FcγRs come together






NK
FcγR
FcγR
Fc
Fab
gp120
Ag
INFECTED




NK
FcγR
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GRANZYMES
PERFORINS
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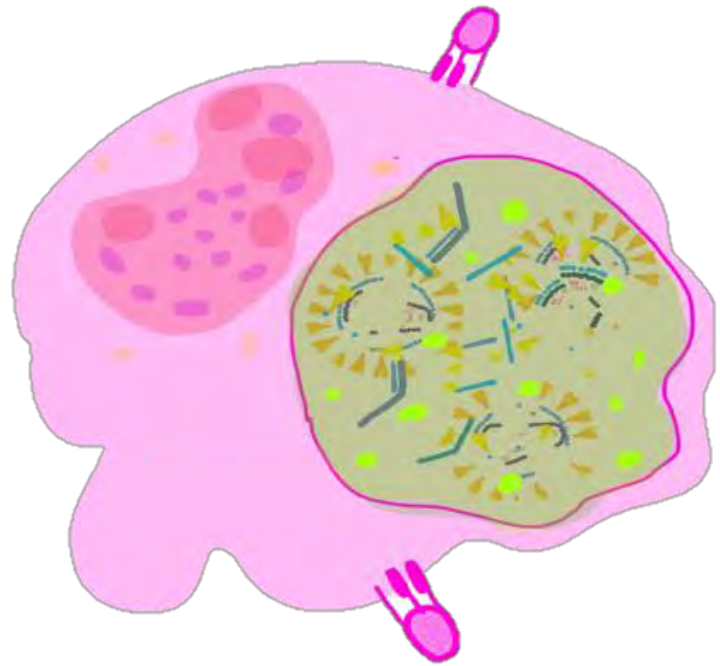
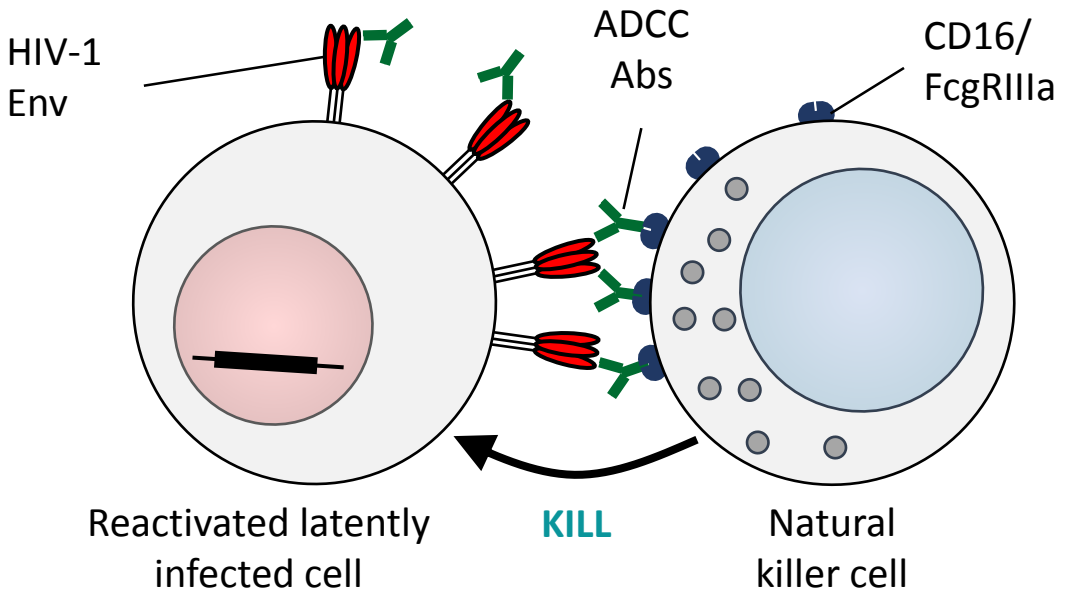


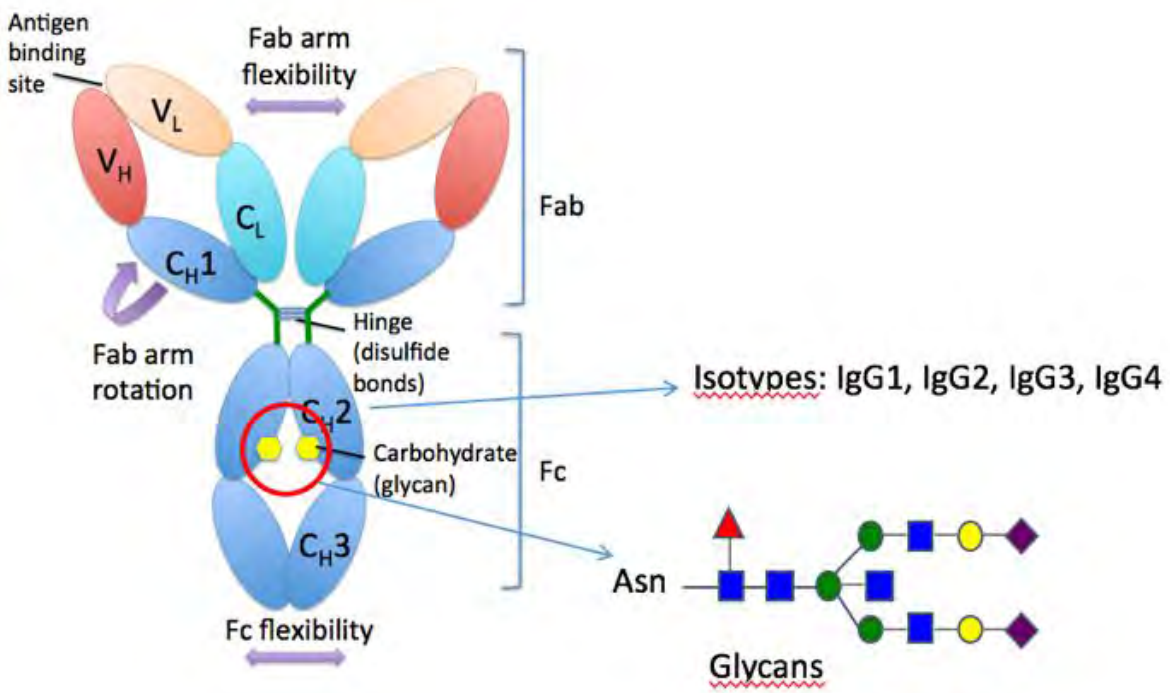
3 way interaction

1. Infected cell membrane presenting Env
2. Antibody
3. Innate effector cell

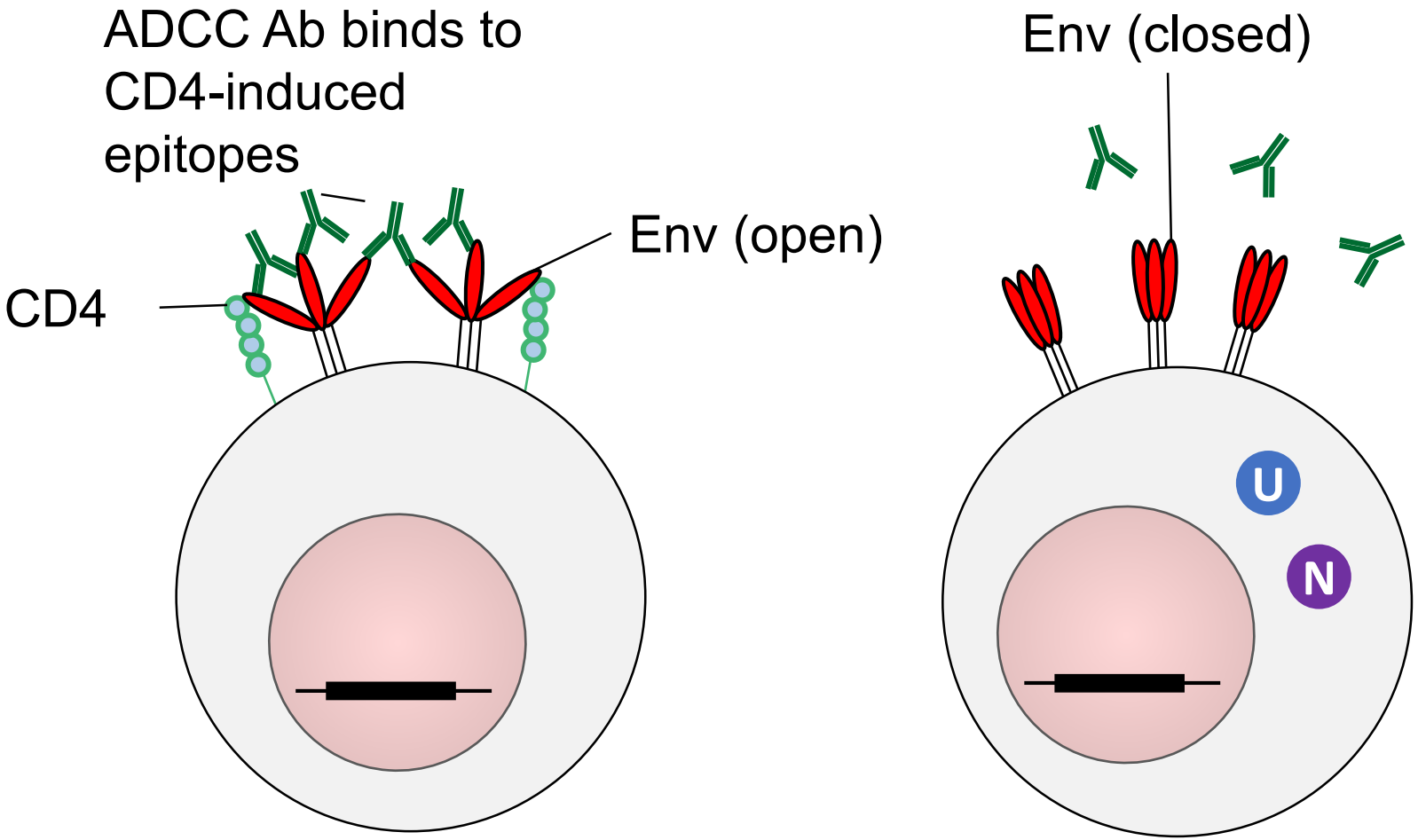


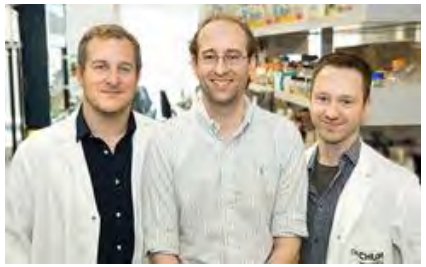
The antibody

- Specificity
- Fc
- Influence of the binding of other antibodies and molecules



Antigen presentation for ADCC:
CD4-downregulation by Vpu and Nef protects infected cells from ADCC





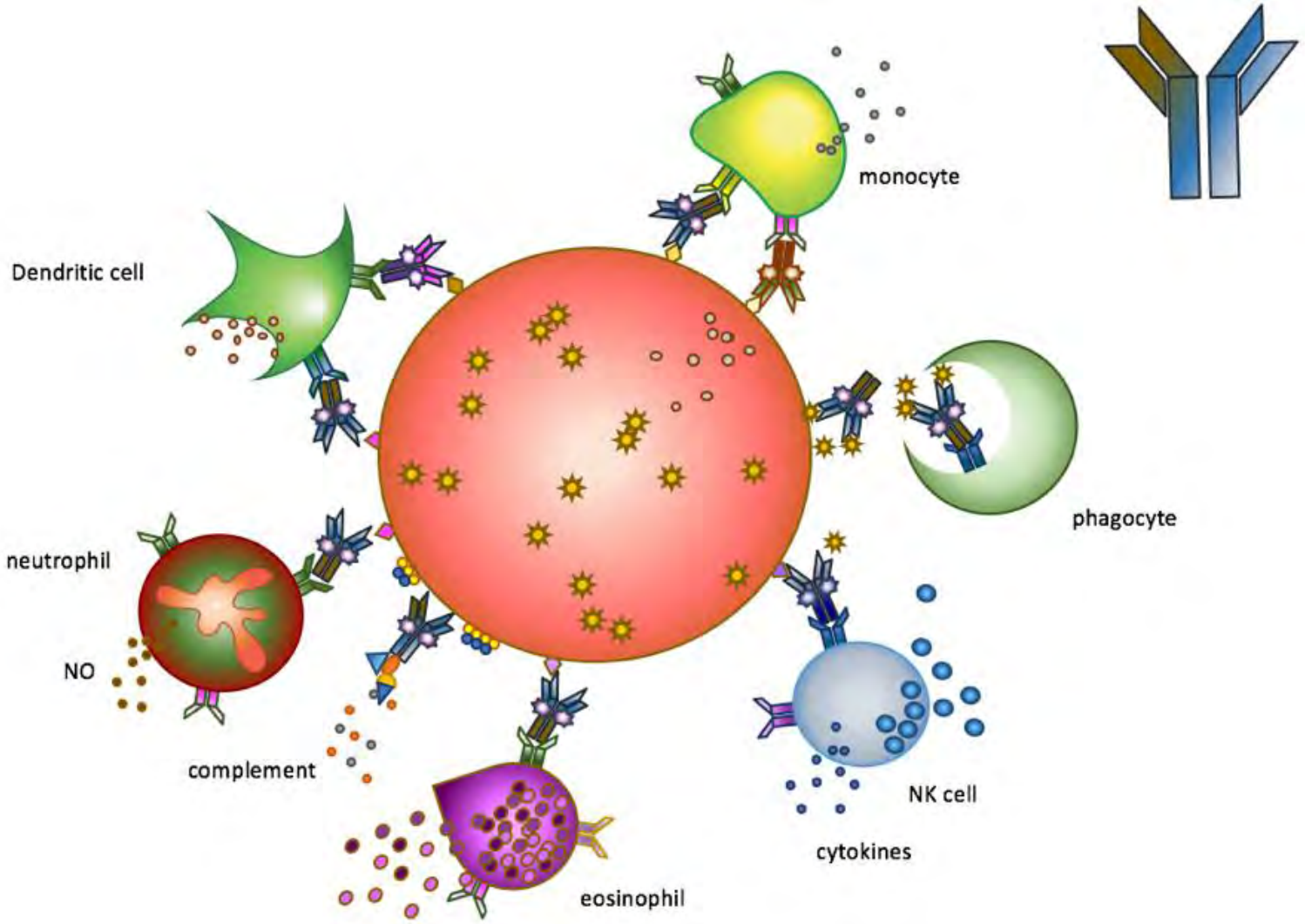
Veillette et al. 2014. J Virol **88:**2633-44.
Pham et al. 2014. Retrovirology **11:**15.
Veillette et al. 2015. J Virol **89:**545-51.

Effector cells

Taking polyfunctionality to the next levels

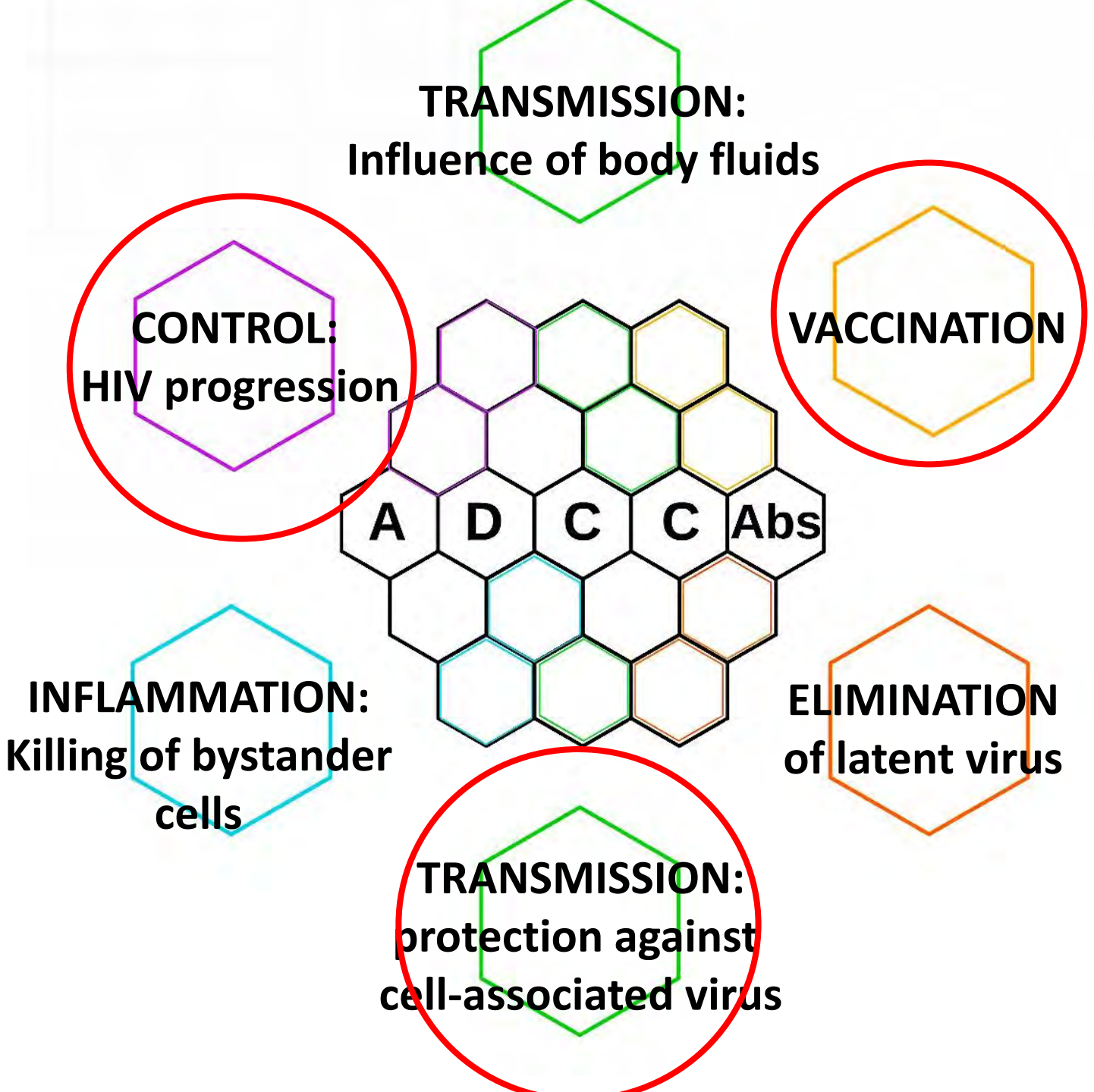
- Multiple cells
- Multiple functions
- binding multiple Fc receptors
- Multiple assays

"systems serology"



Chung et al Cell 2016

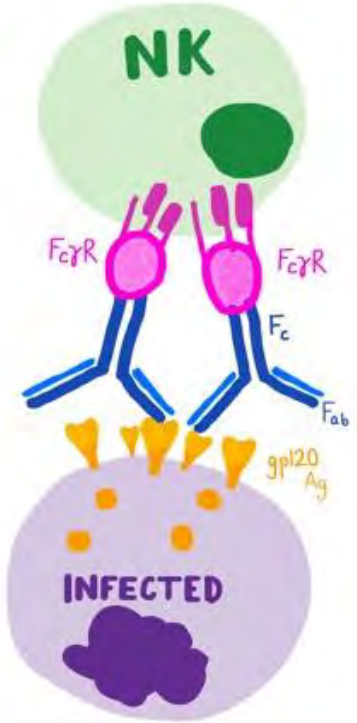
Potential roles for ADCC antibodies

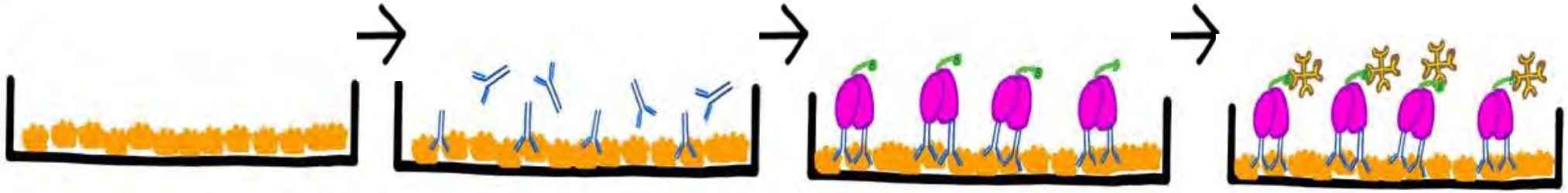


ADCC in vaccine trials

- RV144 suggested a role for V1V2 antibodies and ADCC in protection
- Focus of future efficacy trial work
- Multiple assays can be done - which are the key ones to study on precious samples?
- Reproducibility of assays across labs is a big issue – increased difficult with assays using live innate effector cells
- Key step is the cross-linking of Fcγ Receptors

FcR Dimer assay





Coat plate with HIV-1 protein

Add RV144 plasma

Add biotinylated FcyR dimer

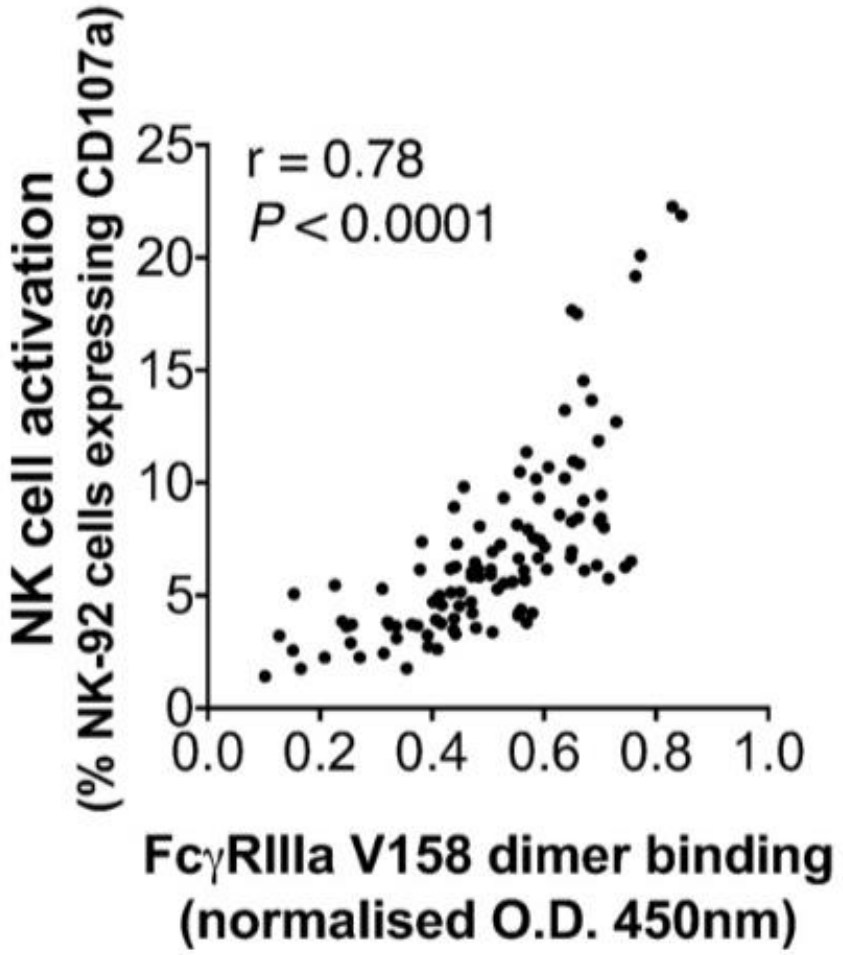
Add Streptavidin-HRP

Read absorbance at 450nm

Wines B, Kent SJ et al. Dimeric FcγR ectodomains as probes of the Fc-receptor function of anti-Influenza Virus IgG. *J*

Bruce Wines, Mark Hogarth

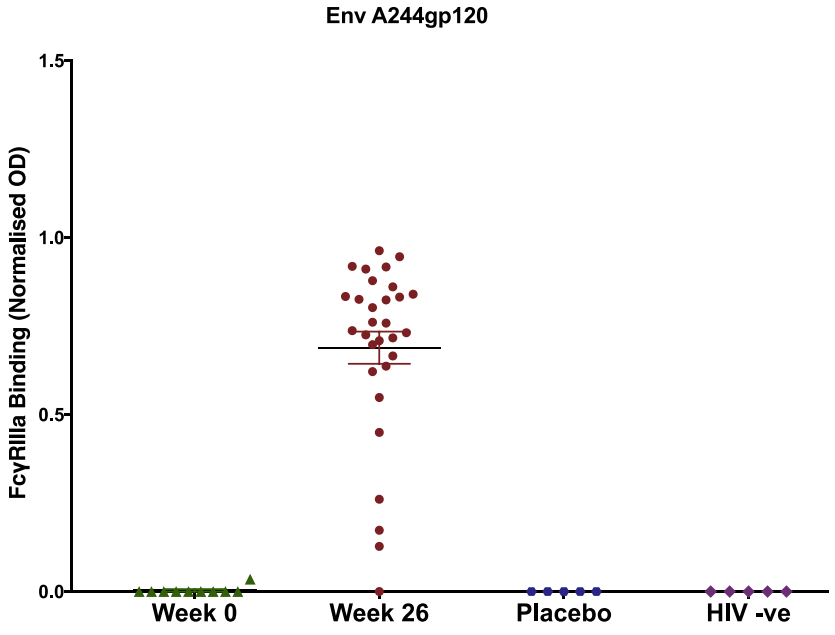
FcγR dimer binding for influenza correlates with functional assays but often more sensitive



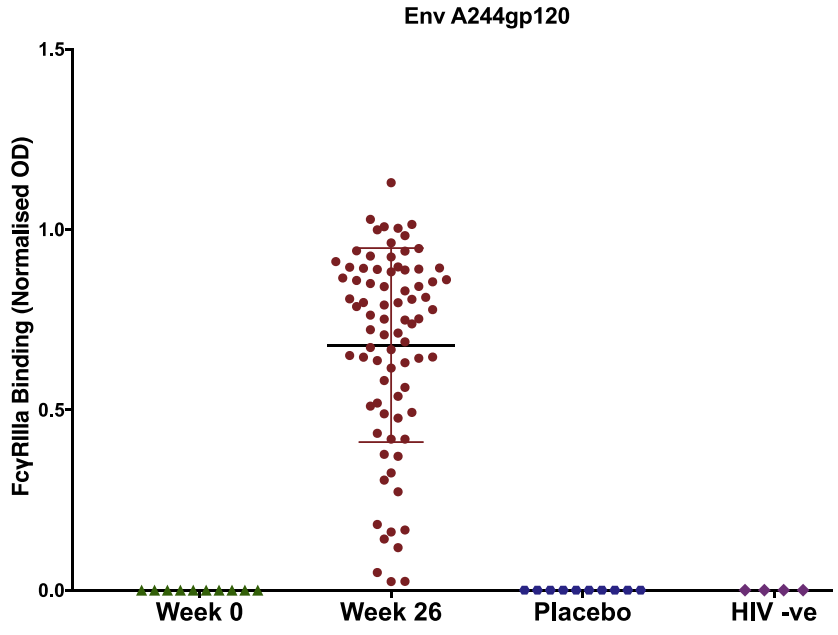
Kristensen et al J Virol 2016, Vanderven et al Ebiomedicine 2016

FcγRIIIa Dimer ELISA on RV144 samples

Test cohort n=30



Validation cohort n=80

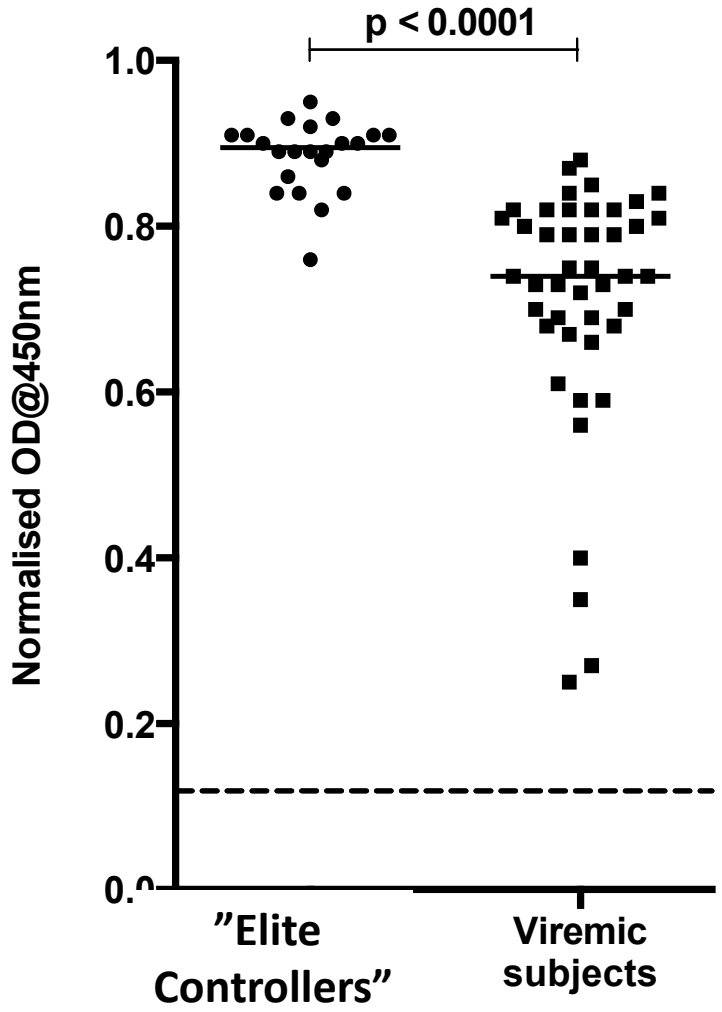


Milla McLean

Control of HIV progression by ADCC

- Considerable data supports a role for ADCC antibodies in partial control of HIV progression

Fcγ-receptor IIIa dimer binding



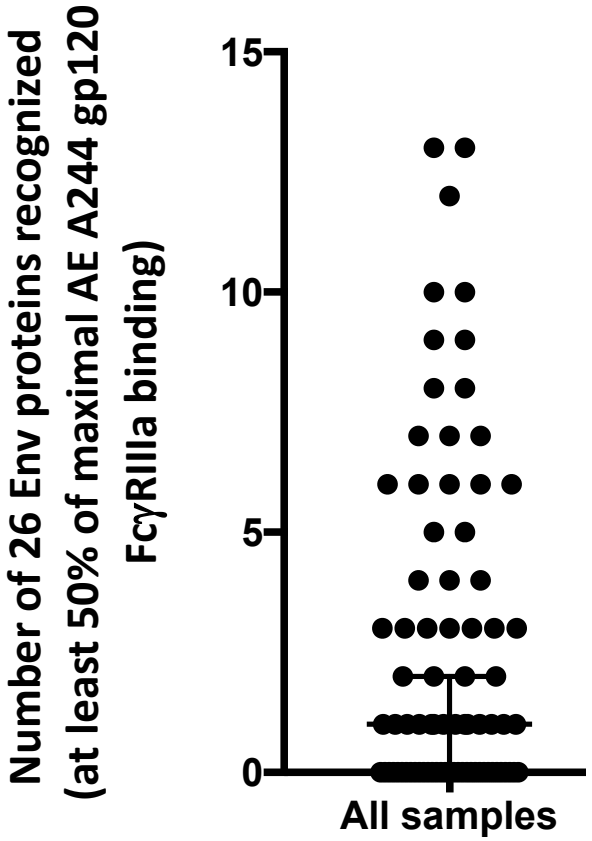
Vijaya Madhavi

So how does V1V2 antibodies align with overall breadth of FcγRIIIa binding antibodies?

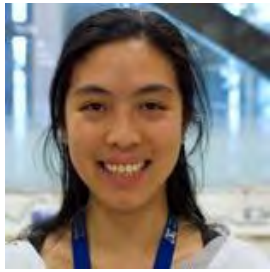
How to classify "breadth" in FcγRIIIa binding antibodies in polyclonal vaccine serum?

Number of 26 Env strains recognized at least 50% of maximal response to vaccine gp120

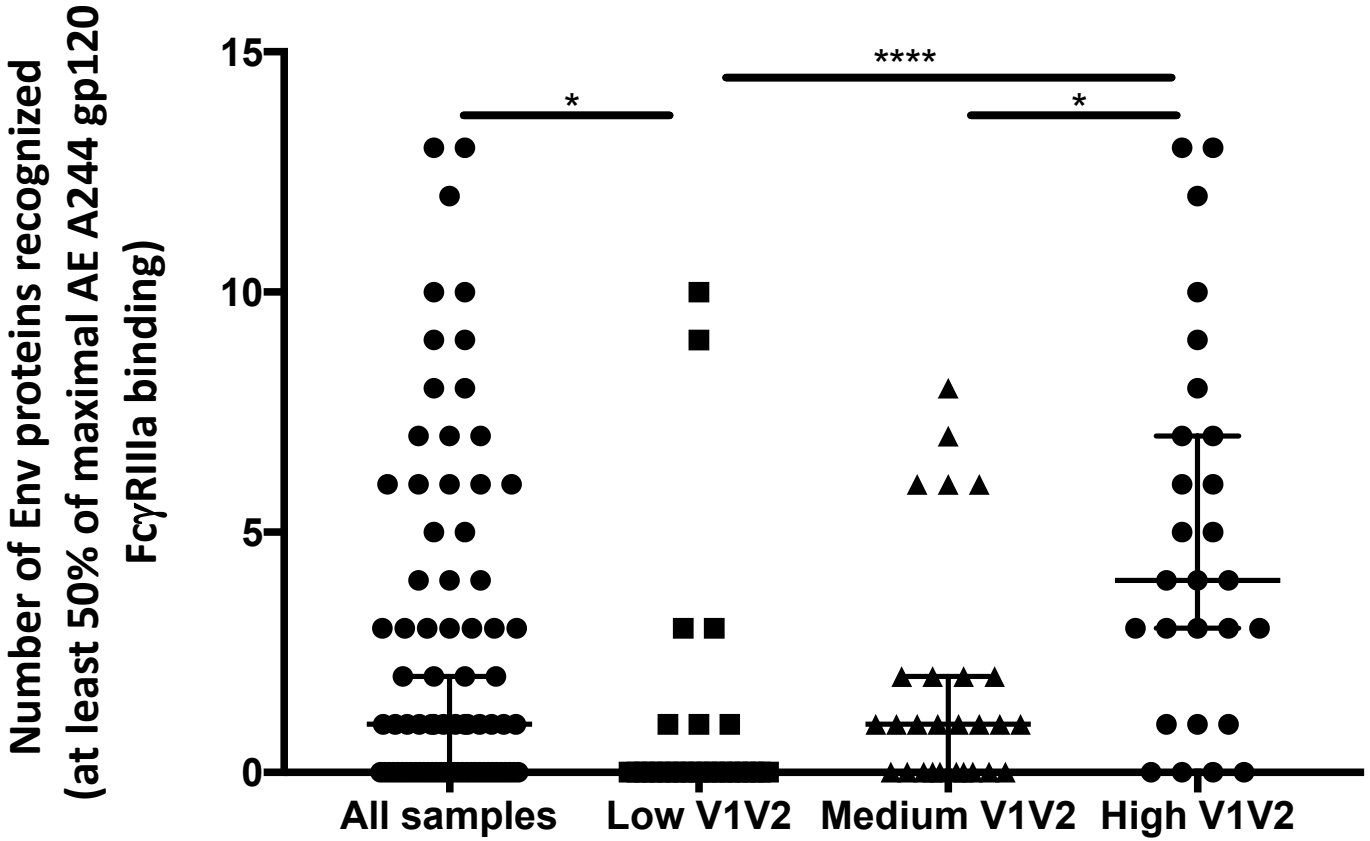
Overall ADCC breadth from RV144 vaccine is modest



Amy Chung



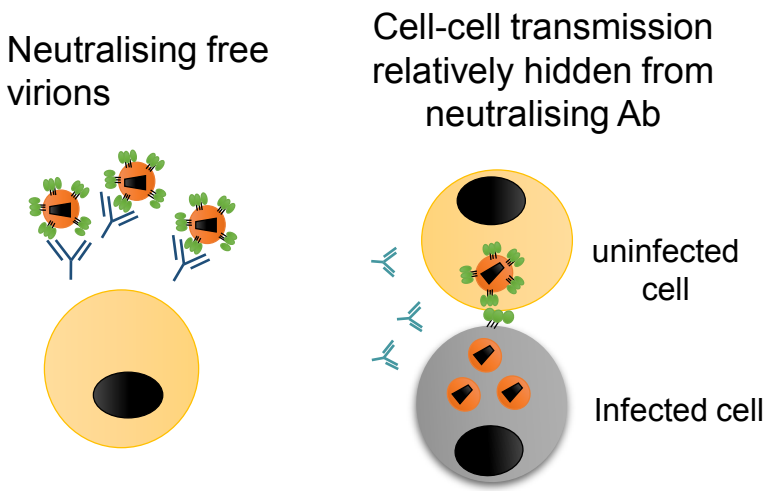
anti-V1V2 binding FcγRIIIa correlate with overall ADCC breadth



Conclusions

- Fc receptor dimer ELISA allows high-throughput analyses of “ADCC”
- Reproducible and correlates well with functional cell-based assays
- Allows an assessment of ADCC breadth
  - Modest in RV144 trial
  - Correlated with V1V2 antibodies
  - Breadth may be important in protection in the field

The problem of cell-to-cell transmission



- Some evidence that cell-cell transmission of HIV could be common – supported by in vitro data and animal models
- Passive Nab transfer studies support a role for ADCC functions in protection against cell-free challenges (Hessell et al Nature 2007)

Matt Parsons

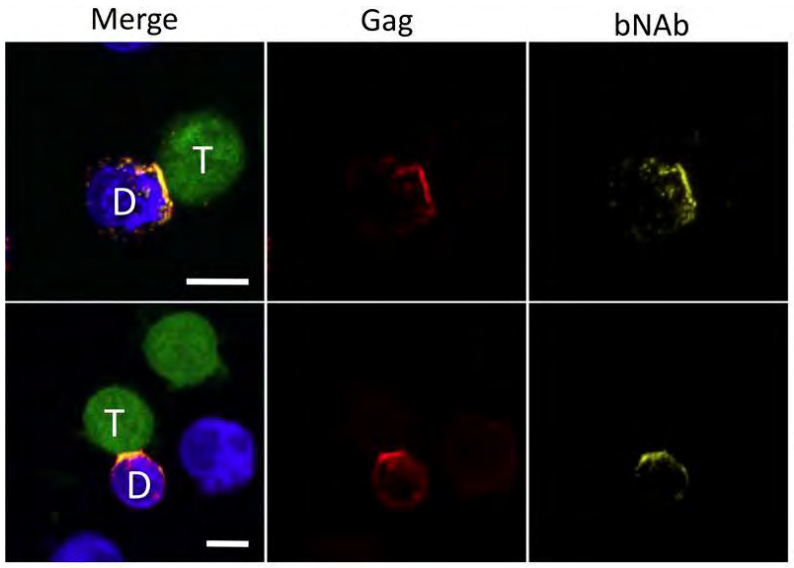
Science Translational Med 2017

Some Neutralising Ab can inhibit cell to cell transmission in vitro?

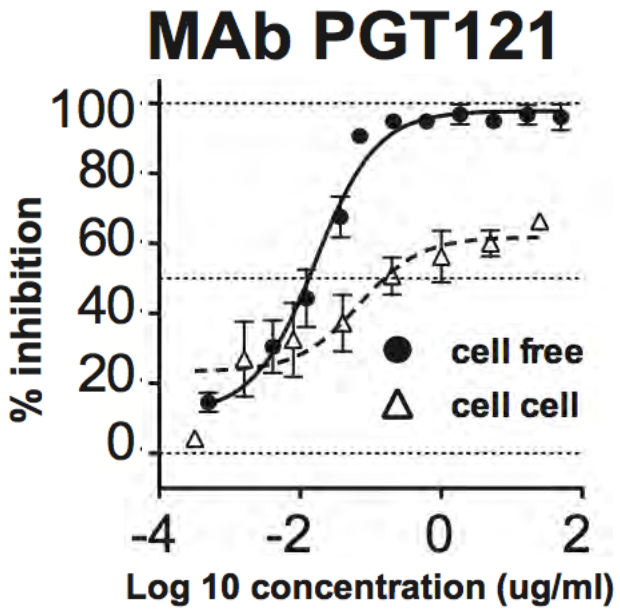
- Receptors and HIV antigens form a virological synapse during cell to cell transmission

- Some neutralising Ab can bind the at the synapse and, in some models (but not others) inhibit virus spread.

- What happens in vivo?



Neutralising Ab binding at the virological synapse of cell-cell transmission

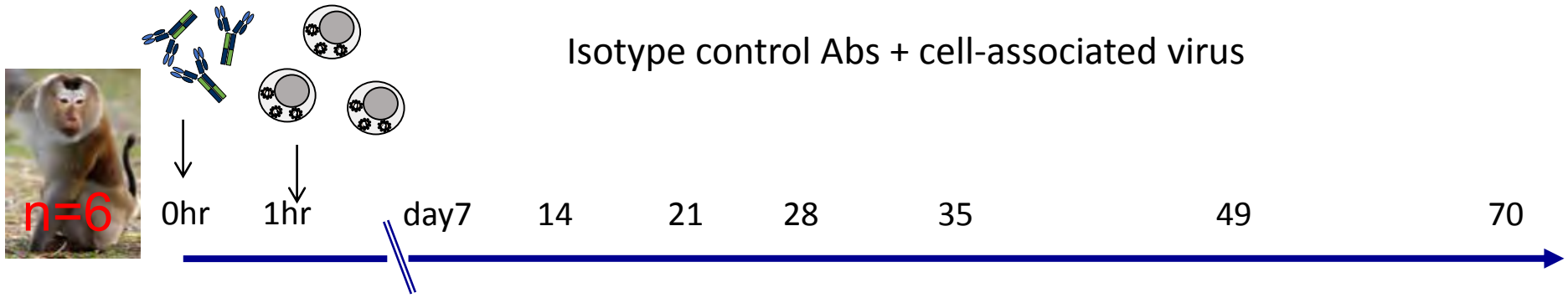


Malbec M., et al, J Exp Med 2013, Gombos et al J Virol 2015, Li et al J Virol 2017

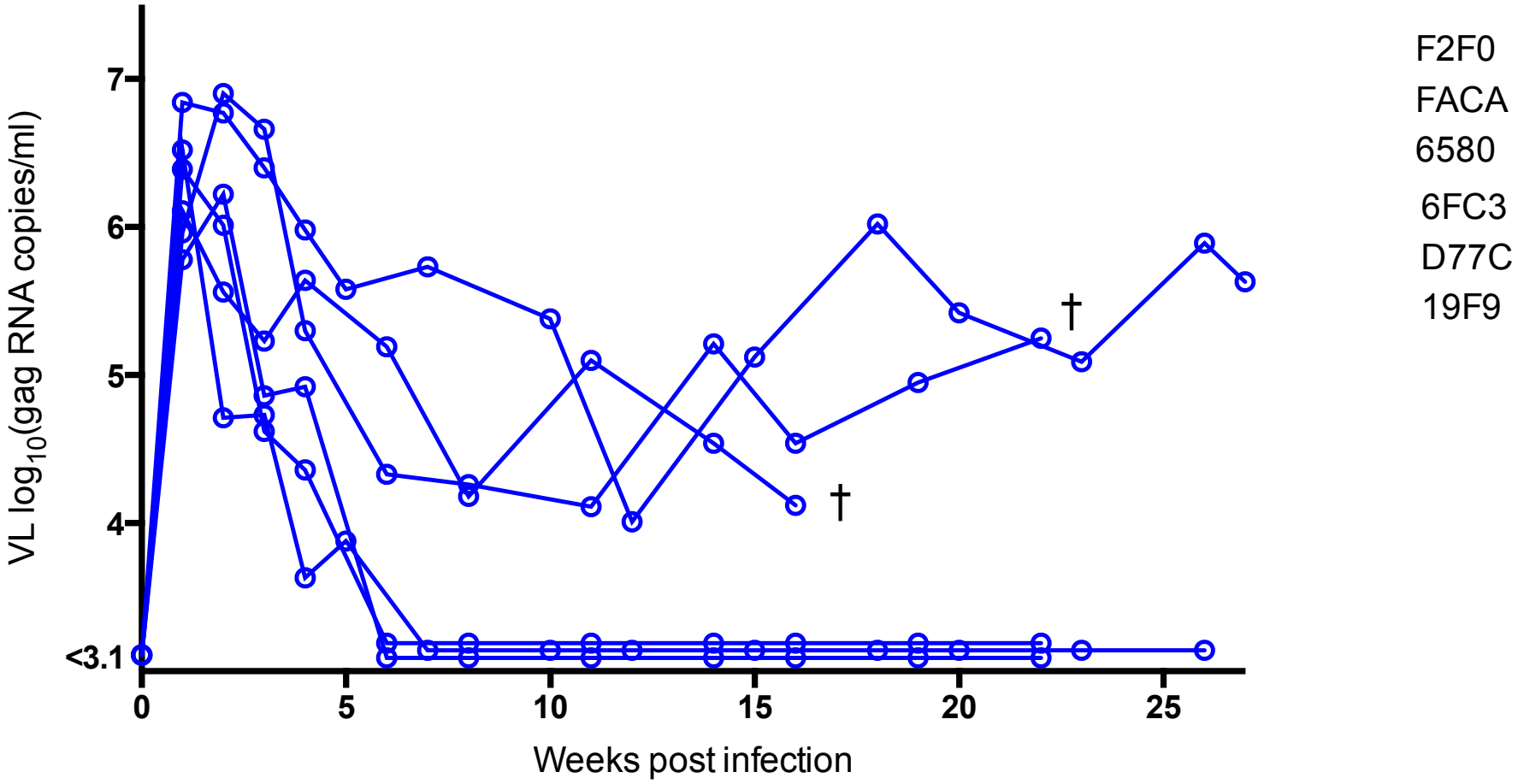
Cell-associated SHIV transmission model

- Developed SHIV model
- IV infusion of $25x10^6$ $SHIV_{SF162P3}$ splenocytes from animal with acute SHIV
- ~1000 animal infectious doses - Robust infection model!

  - 2 naïve animals
  - 4 animals given an isotype control antibody

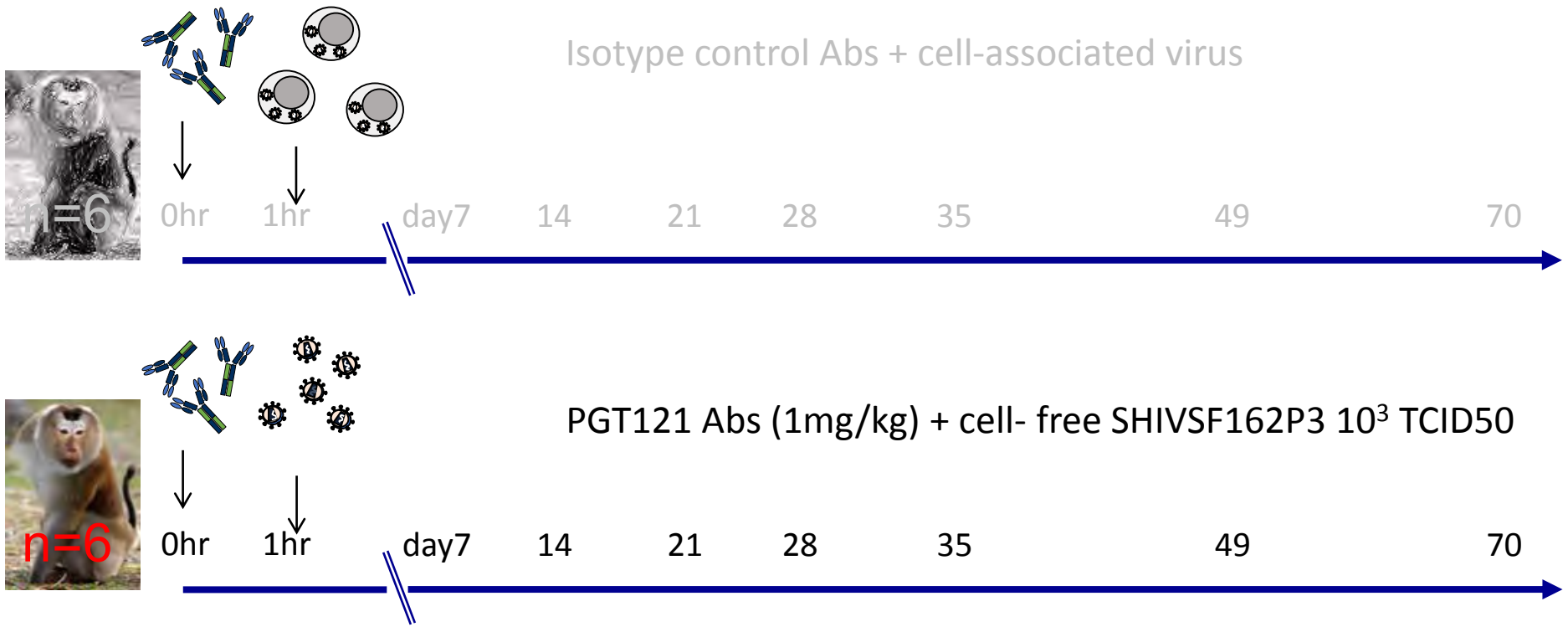


Isotype control animals exposed to cell-associated $SHIV_{SF162P3}$ become infected

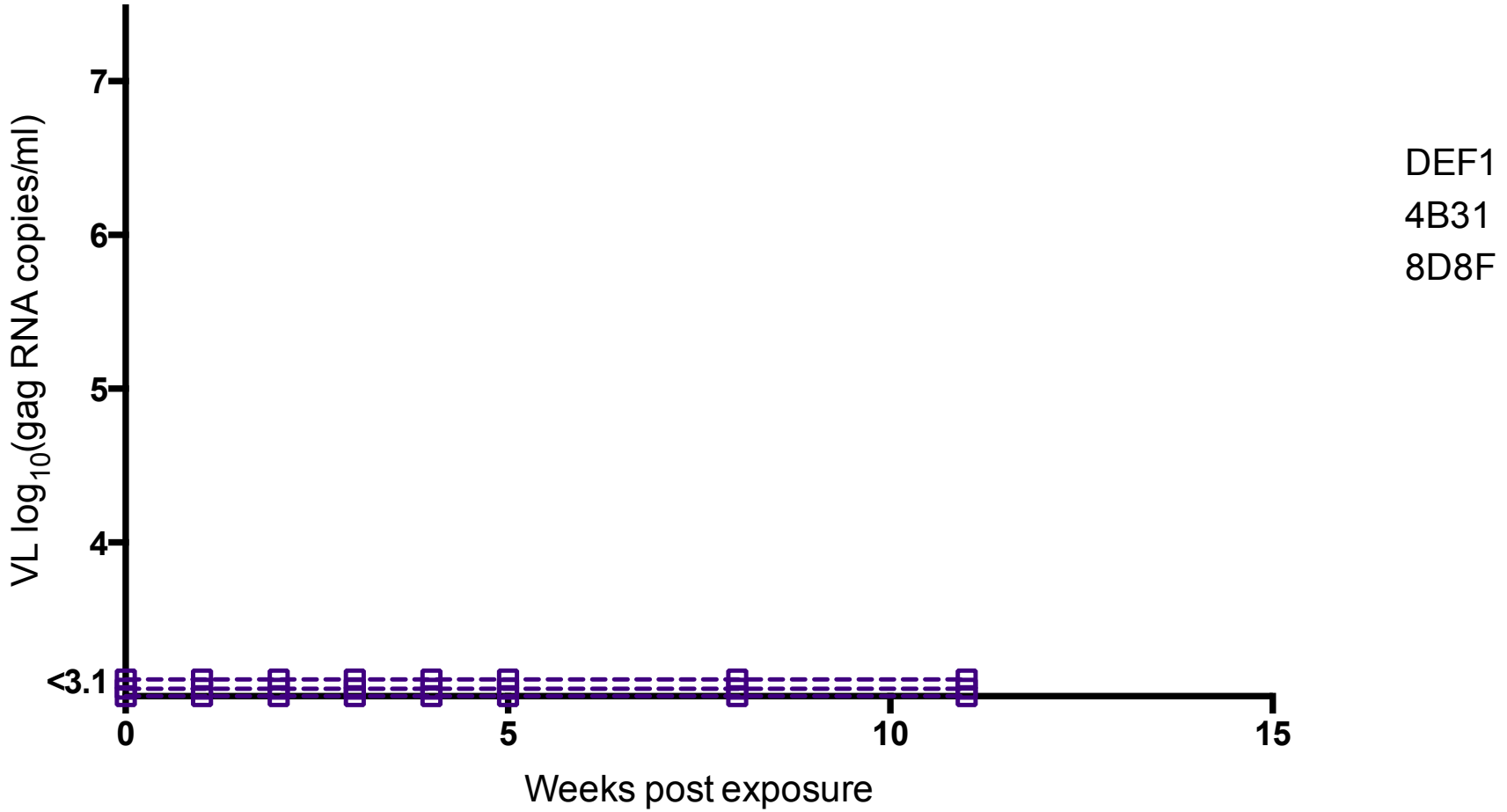


Confirm that **Nab PGT121** protects against cell-free SHIV challenge

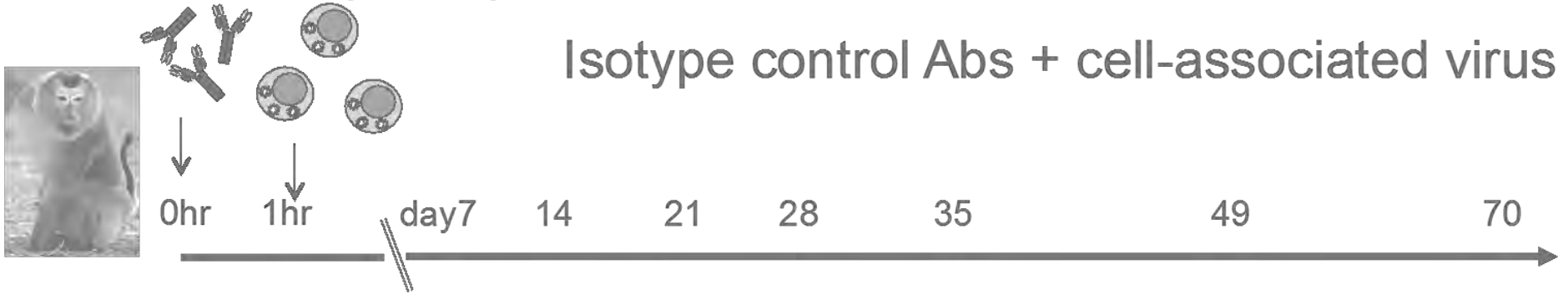
- Protection previous observed in Rhesus macaques with same PGT121 dose (Moldt et al PNAS 2012)

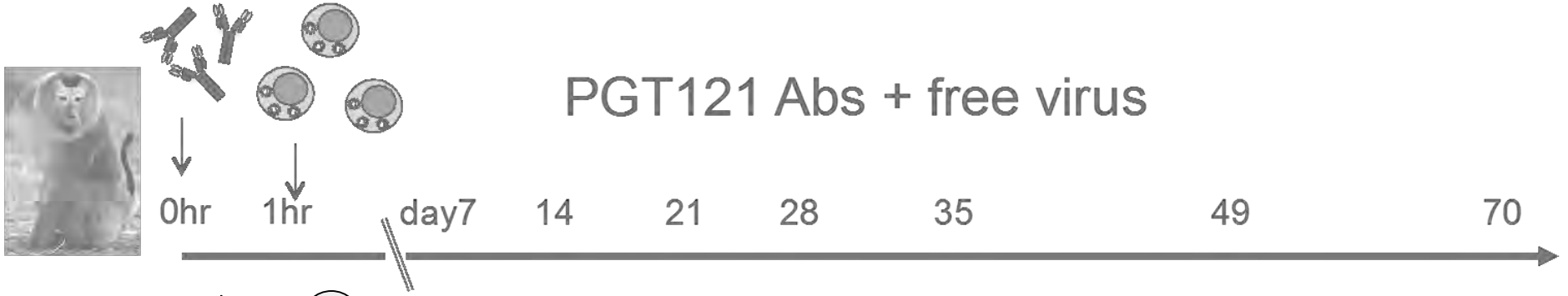


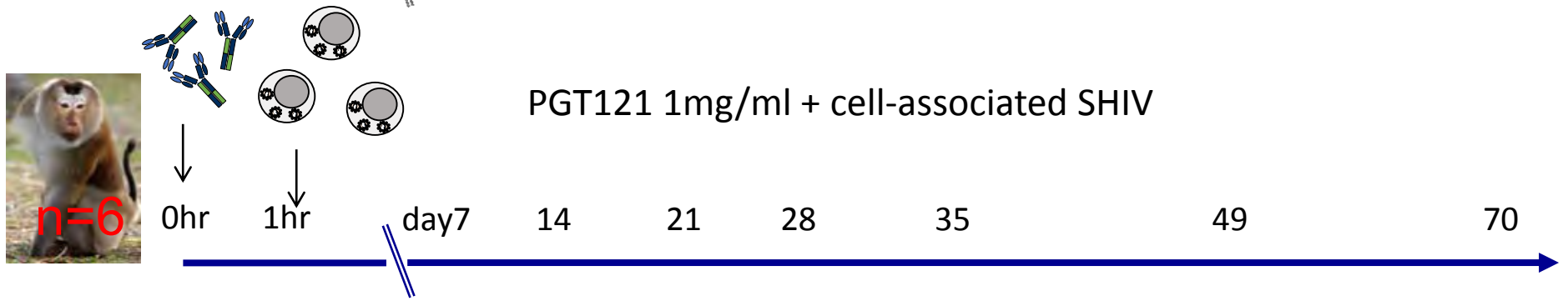
Complete protection by PGT121 from cell-free challenge



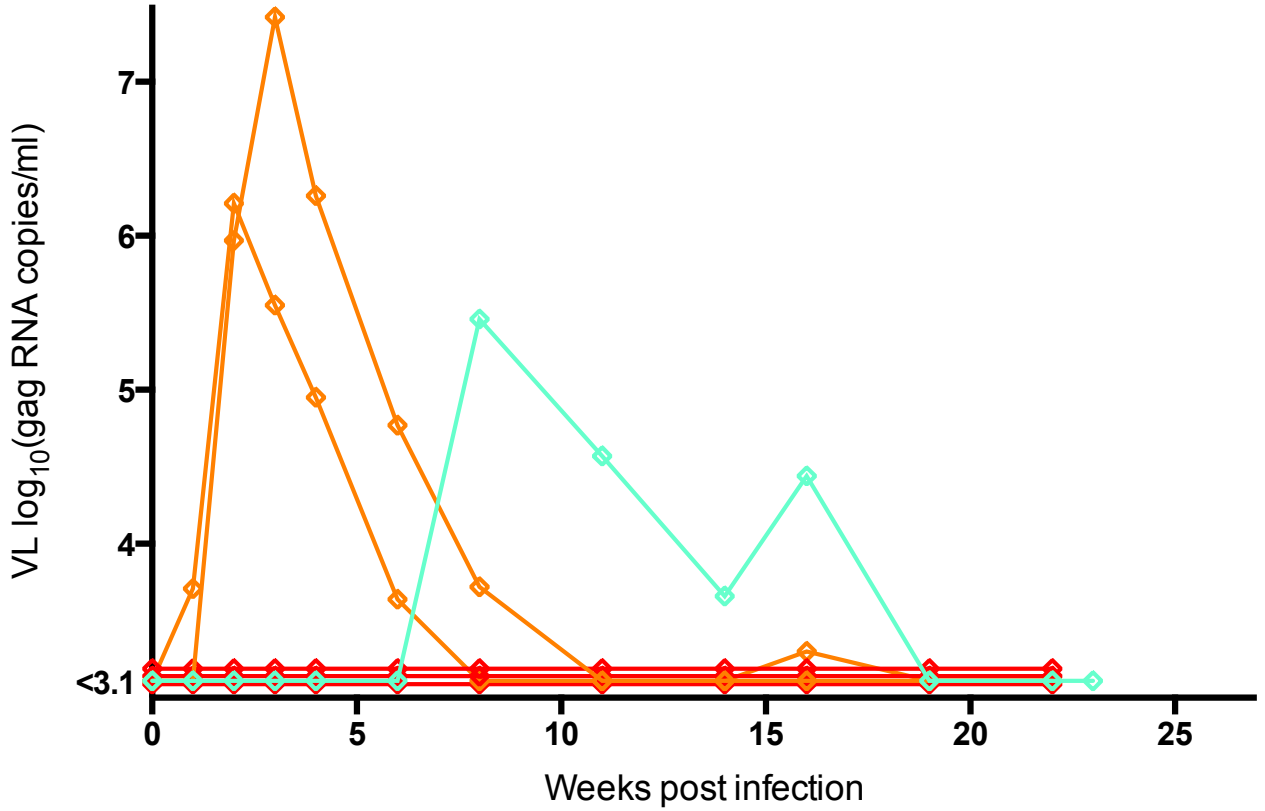
Timeline


Isotype control Abs + cell-associated virus
0hr
1hr
day7
14
21
28
35
49
70


PGT121 Abs + free virus
0hr
1hr
day7
14
21
28
35
49
70


PGT121 1mg/ml + cell-associated SHIV
n=6
0hr
1hr
day7
14
21
28
35
49
70

Viral load in PGT121 animals exposed to cell-associ

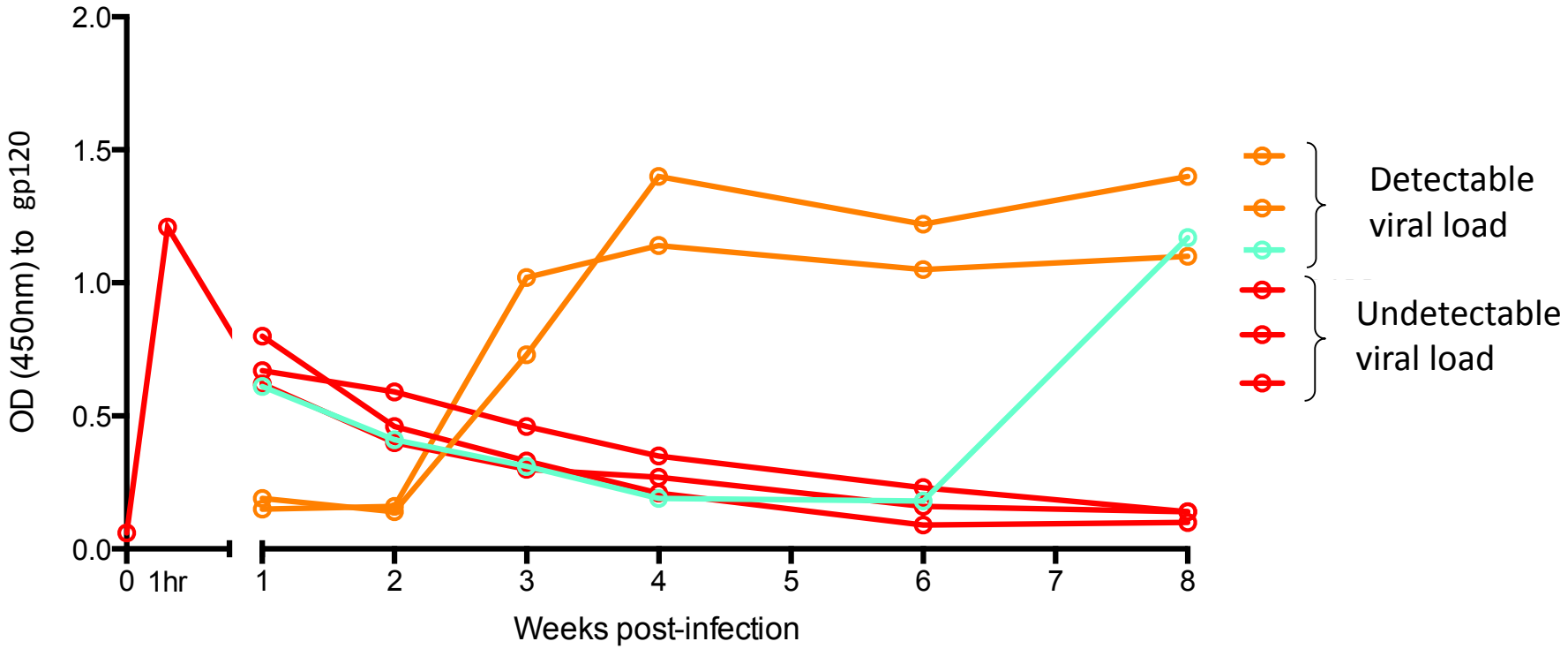


We saw 3 patterns of infection: n=3 total control

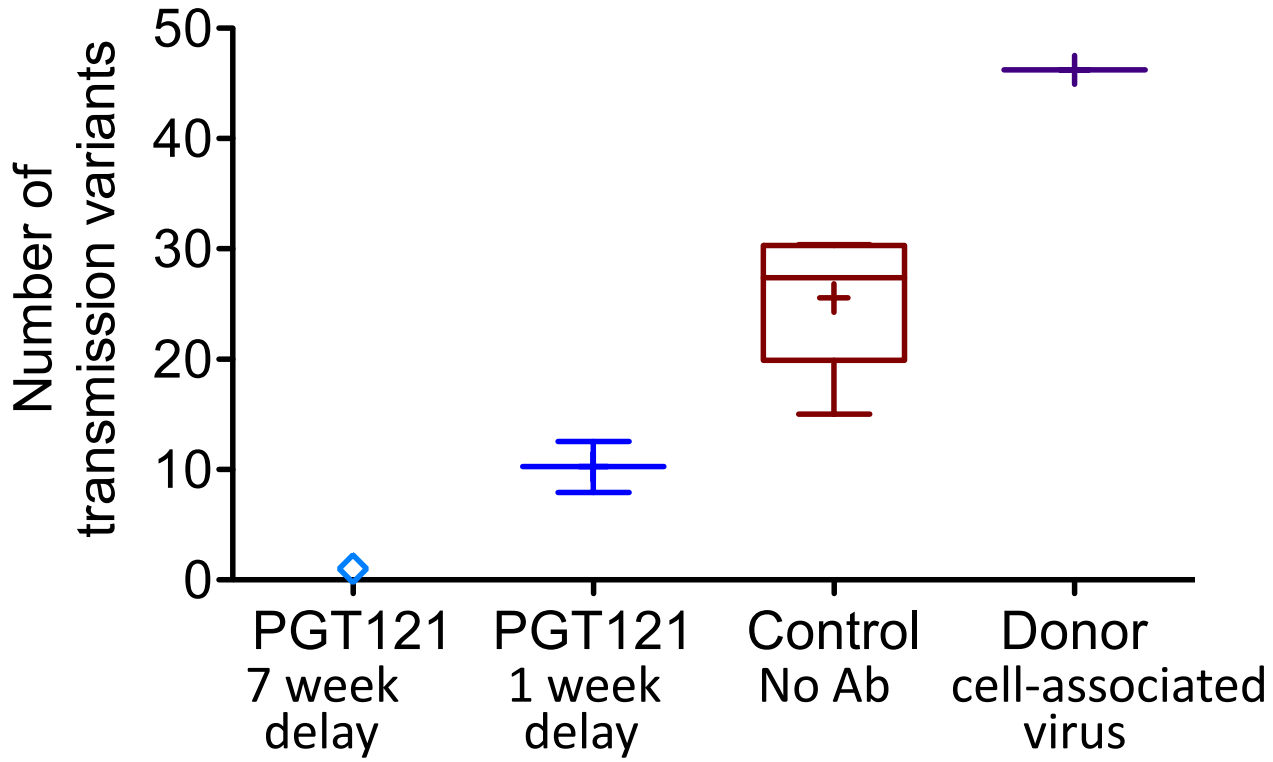
n=2 infection with 1-week delay

n=1 **7-week delayed infection**

Decay of PGT121 levels in PGT121 animals exposed to cell-associated virus



Reduced diversity of breakthrough SHV infection despite BNAb



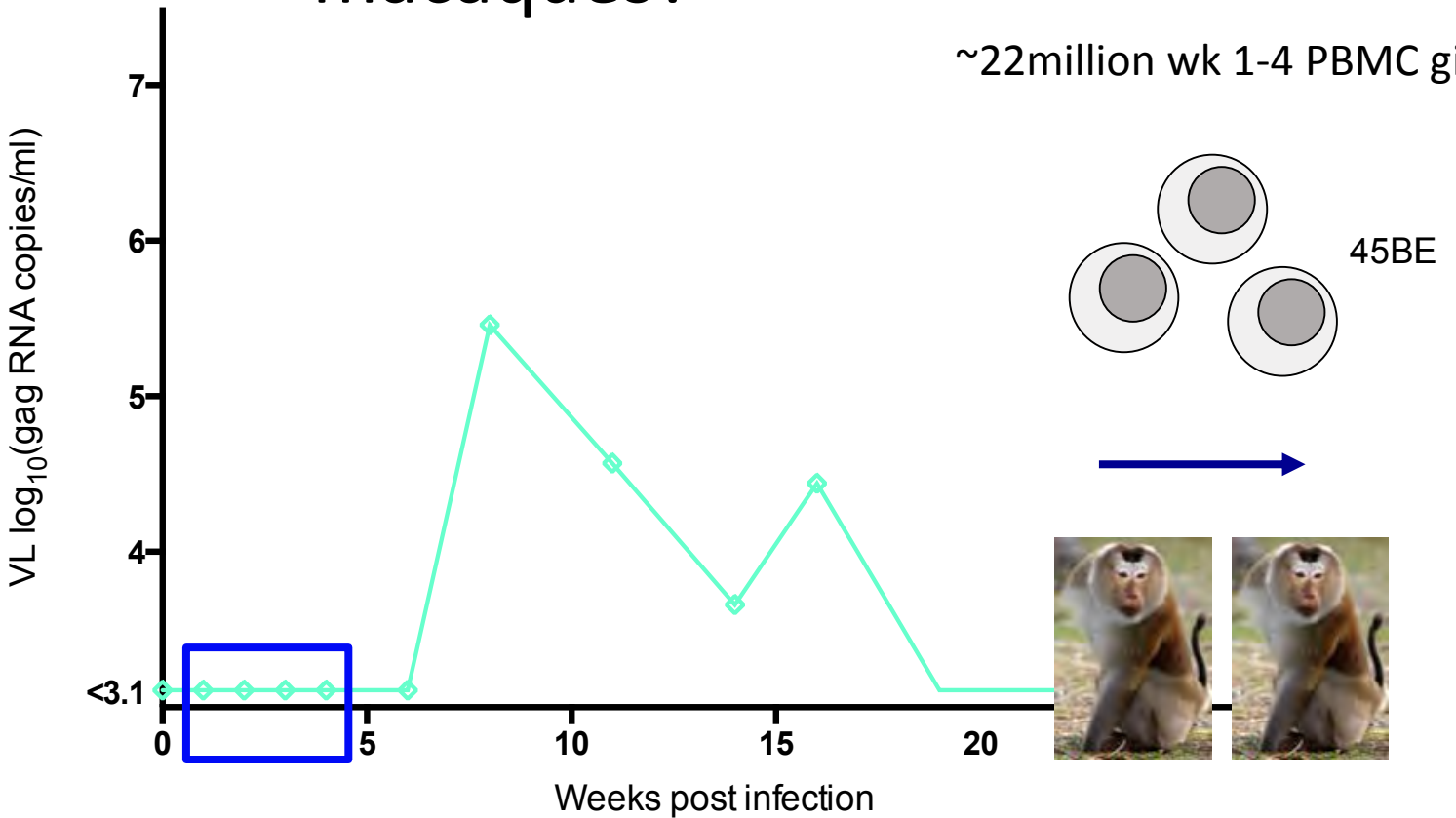
Miles Davenport, Vanessa Venturi, Brandon Keele

Implications

- Bnab only protected a proportion of macaques against a high-dose cell-associated virus challenge
- Break-through occurred with limited numbers of founder viruses as Ab levels declined
  - No evidence of PGT121 resistance
- Possible “occult” virus in tissues relatively hidden from Bnab
- Fc-mediated Ab functions likely to be even more important against cell-associated virus
- Implications for “Antibody-Mediated Protection”
  - **watch out fore excess of infections when the Ab levels decline**

Where was the virus?

- No RNA or DNA detected in blood prior to week 7, including sensitive assays kindly performed by Lifson lab
- Can virus be transferred by PBMC infusion to uninfected macaques?



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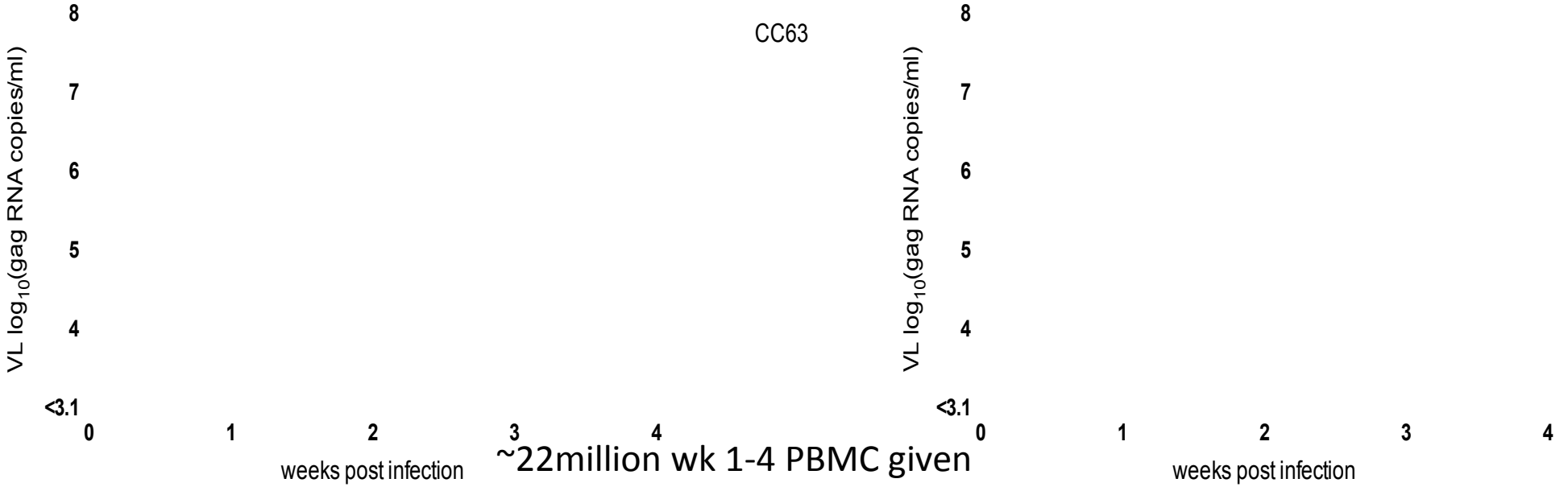
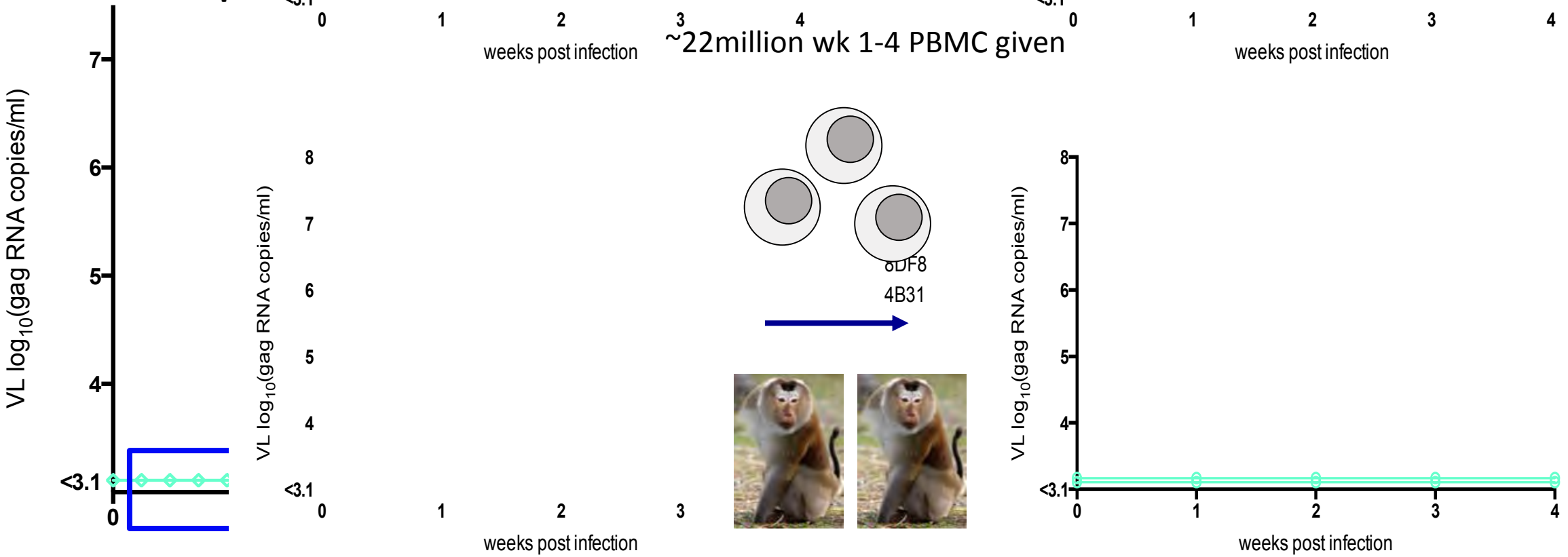


~22million wk 1-4 PBMC given



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Thakshila Amarasena
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Vanessa Venturi
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Tony Kelleher
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Duke University
David Montefiori
Celia Labranche

University of Montreal
Andres Finzi
Jonathon Richard

Thai Red Cross HIV Laboratory
Kiat Ruxrungtham
Torsak Bunupuradah

Wisconsin Primate Center
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Roger Wiseman

Theraclone Sciences
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EAVI2020 EUROPEAN AIDS VACCINE INITIATIVE

HORIZON 2020

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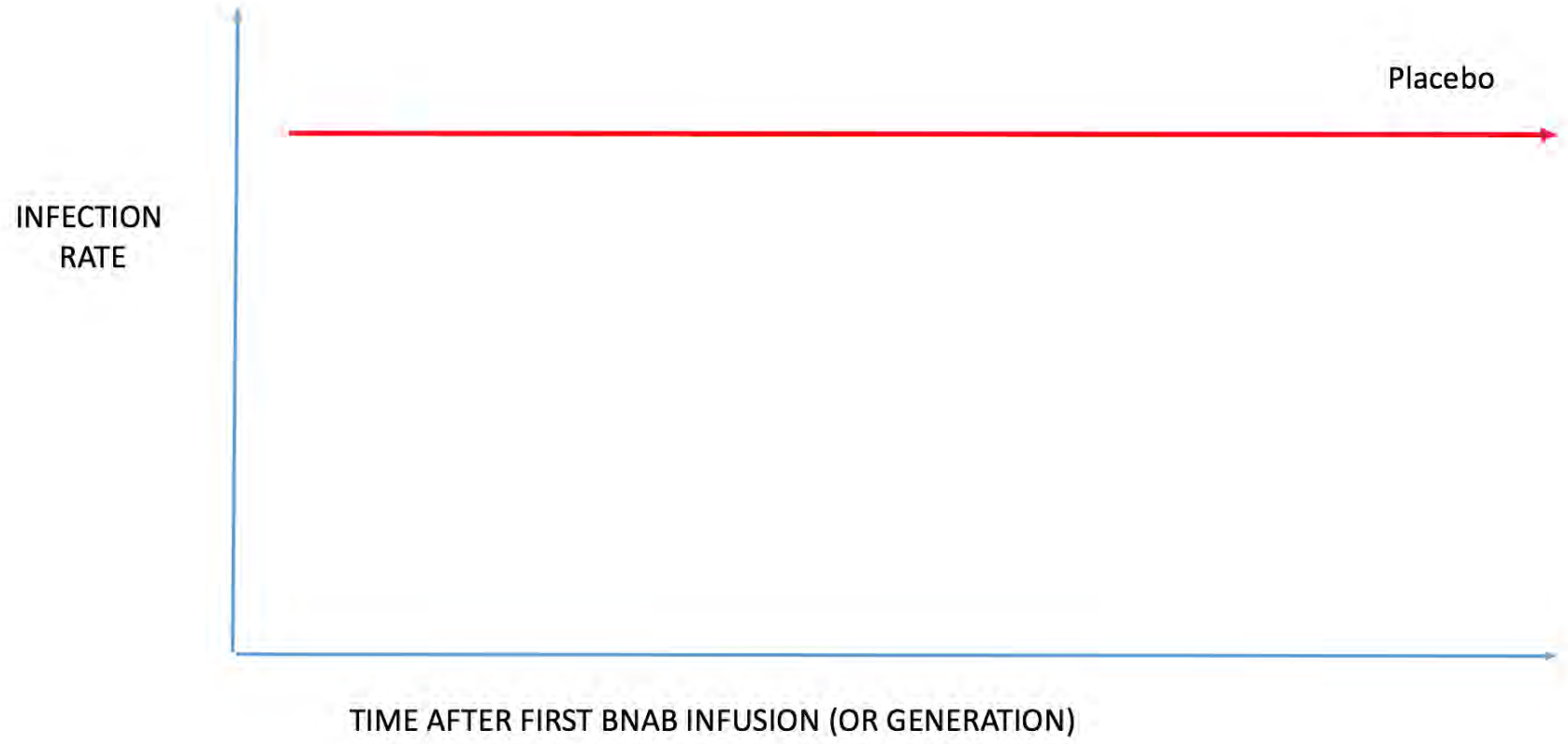
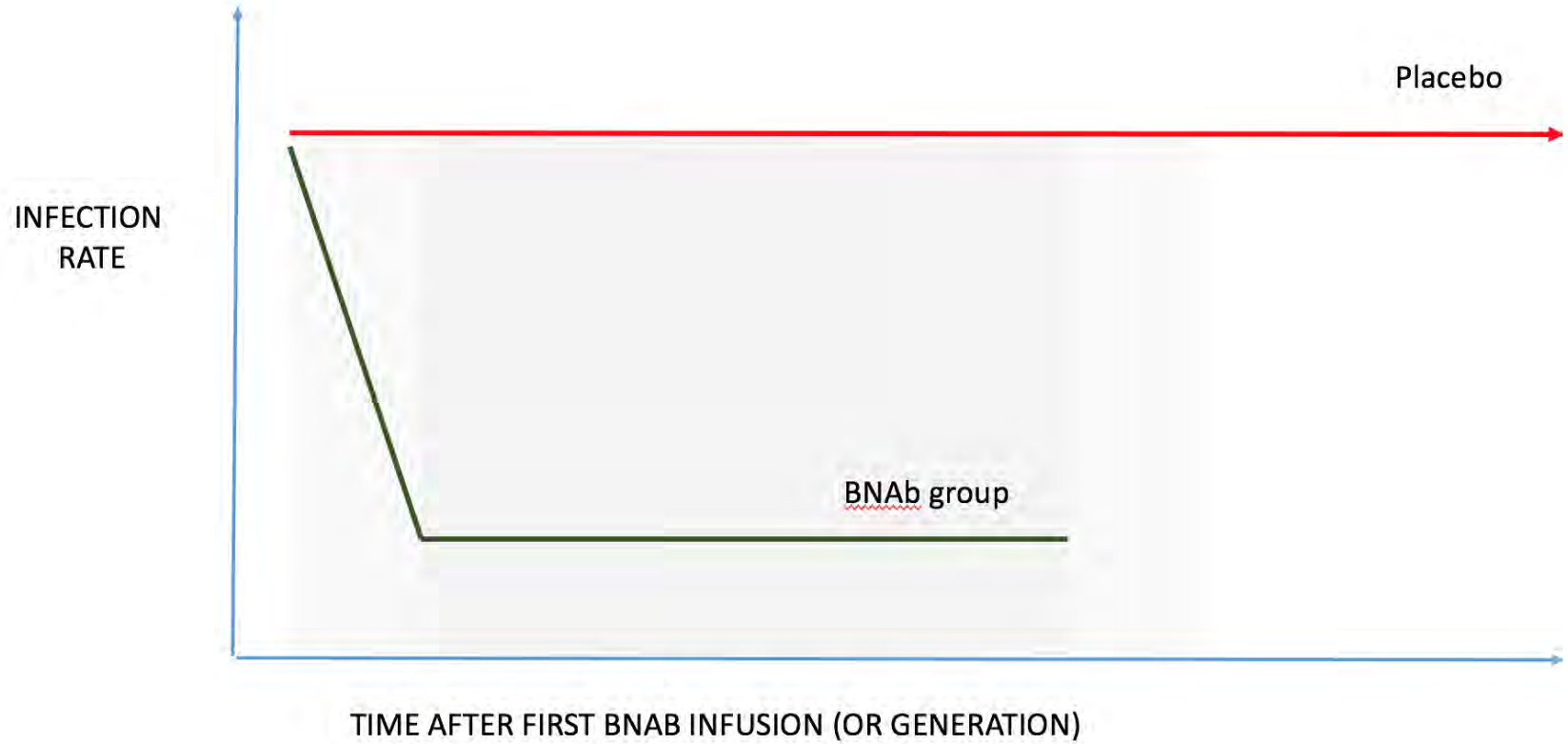
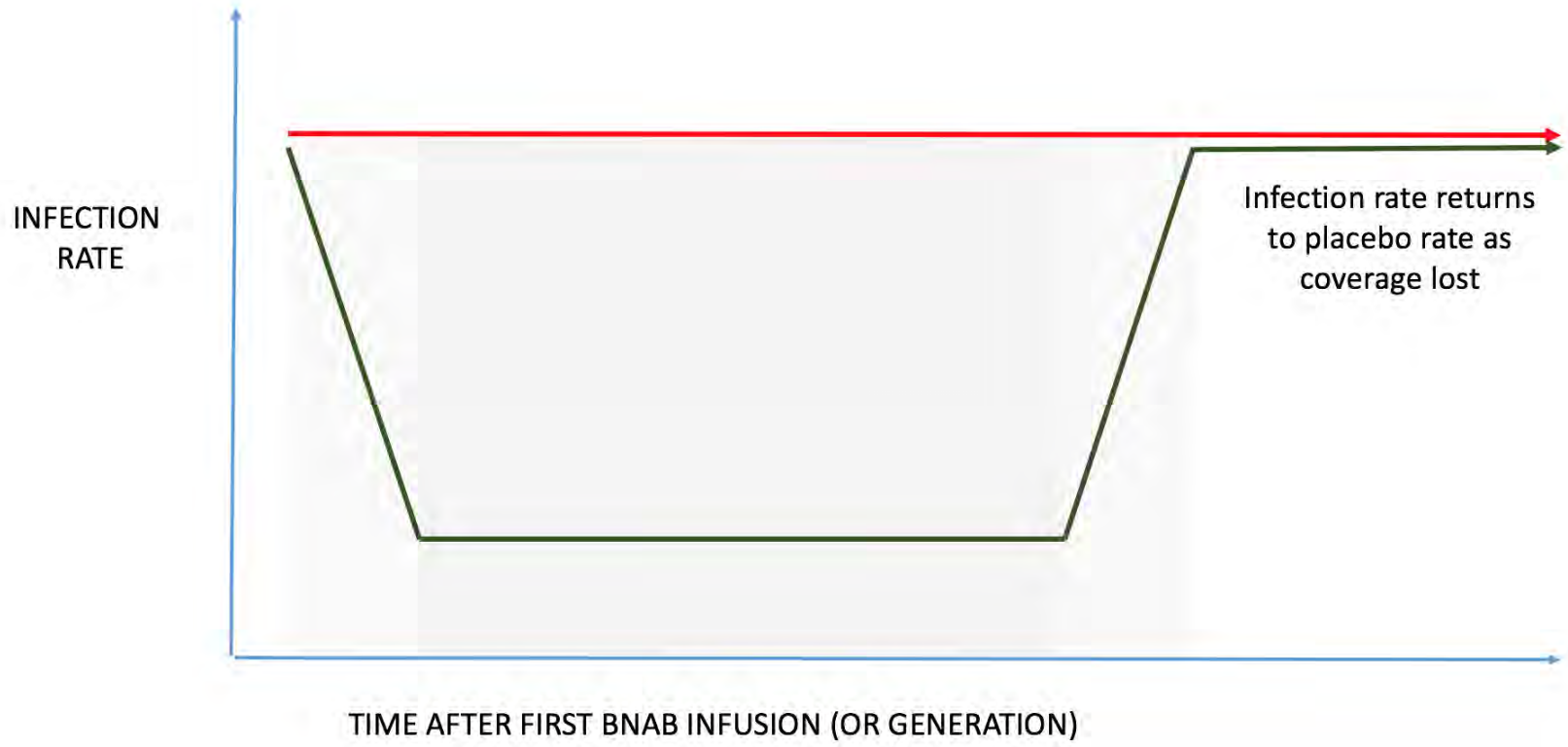
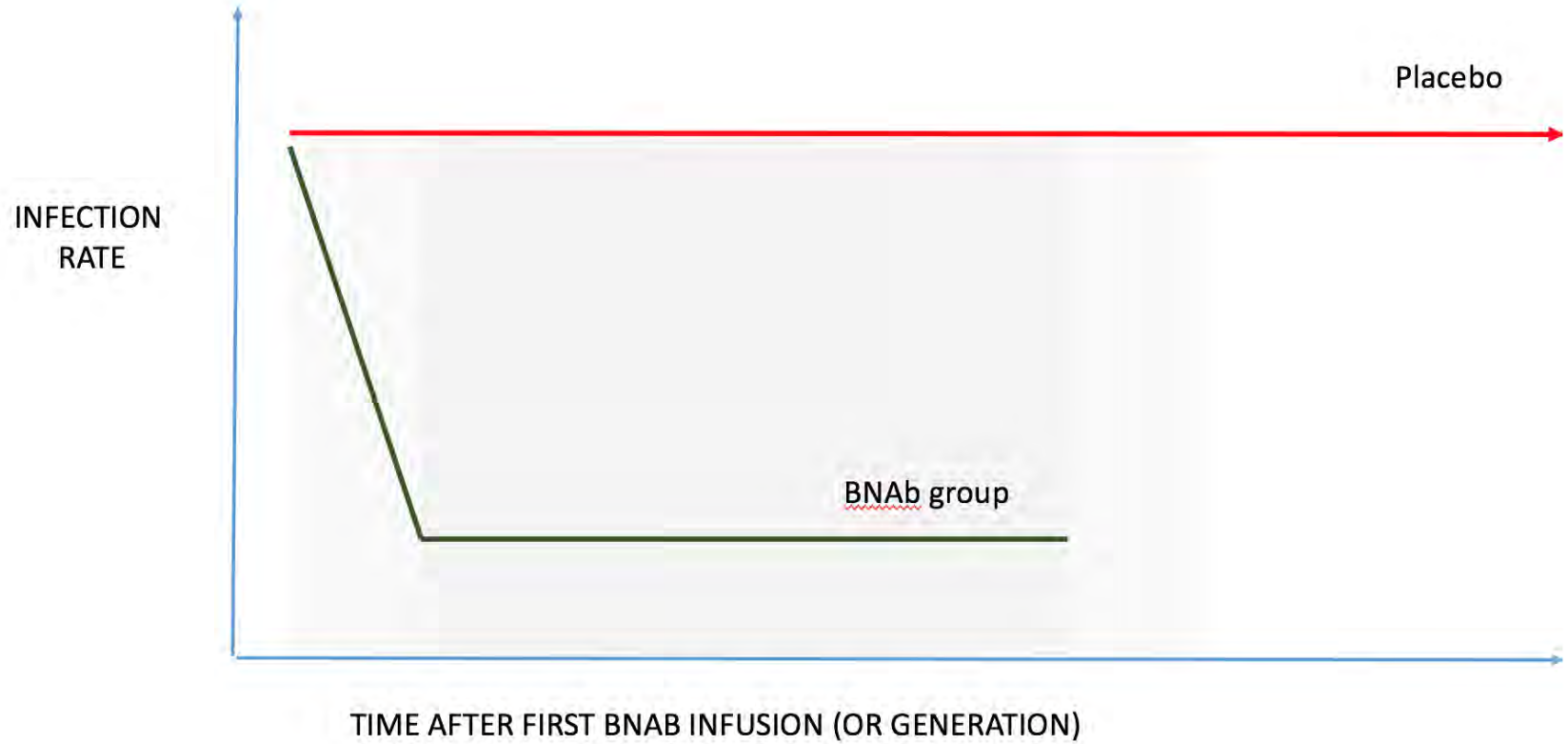
Hypothetical outcome of Passive Bnab transfer studv

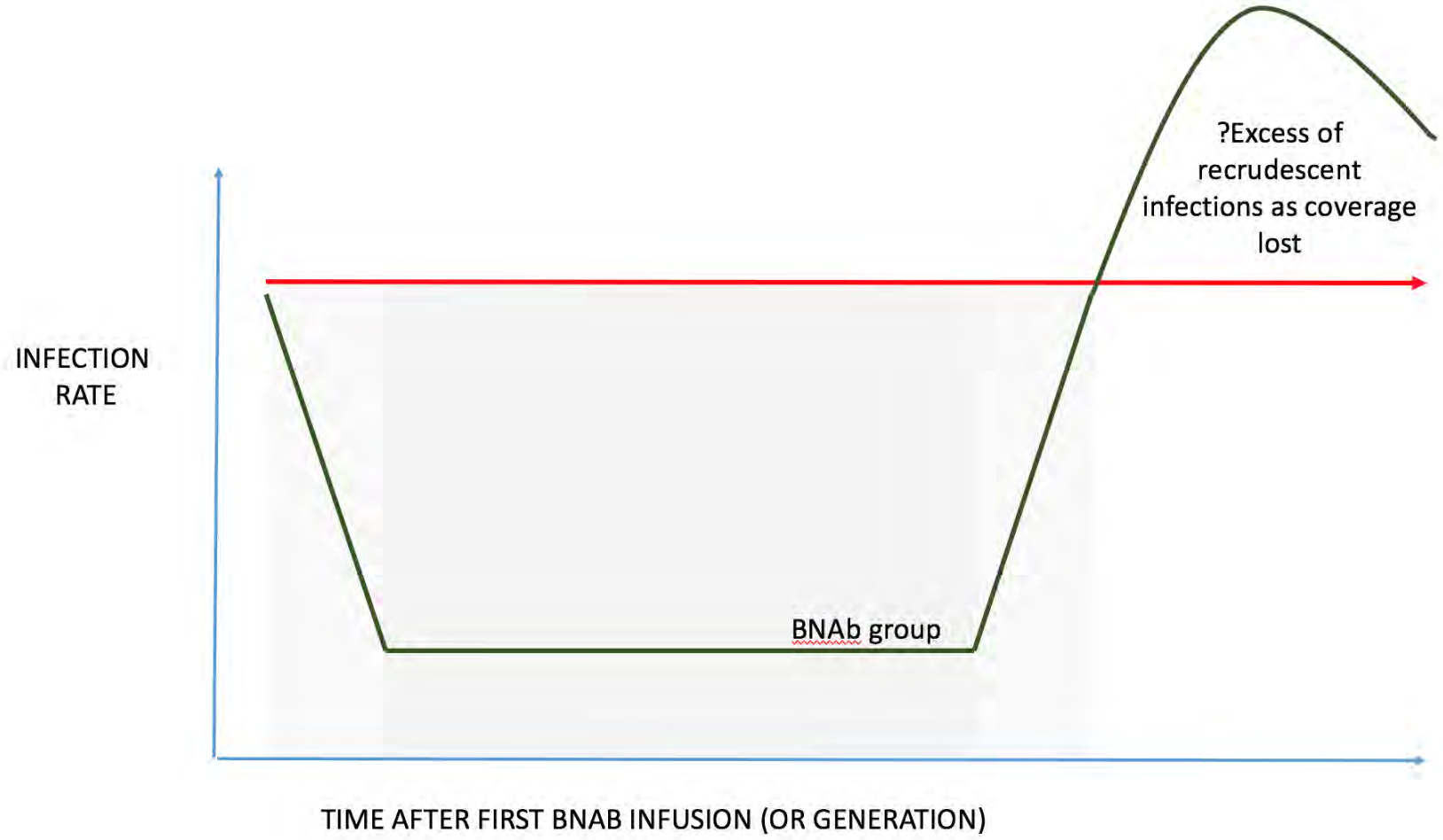



INFECTION RATE
BNAb coverage
TIME AFTER FIRST BNAb INFUSION (OR GENERATION)


Placebo
INFECTION RATE
BNAb group
TIME AFTER FIRST BNAB INFUSION (OR GENERATION)


INFECTION RATE
Infection rate returns to placebo rate as coverage lost
TIME AFTER FIRST BNAB INFUSION (OR GENERATION)


Placebo
INFECTION RATE
BNAb group
TIME AFTER FIRST BNAB INFUSION (OR GENERATION)


?Excess of recrudescent infections as coverage lost
INFECTION RATE
BNAb group
TIME AFTER FIRST BNAB INFUSION (OR GENERATION)

Future Nab based vaccine study concepts

- Look late for recrudescence of infections from virus infected cells when Nab has gone
- In human passive transfer efficacy studies, look for bump in infections early after infusions finished